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Novel Biomarkers for the Monitoring of Chronic Heart Failure

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Novel Biomarkers for the Monitoring of Chronic Heart Failure

In part fulfilment of MD(Res)

At

Kings College London



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BSc(Hons) MBBS MRCP(UK)

King's College London

STATEMENT OF ORIGINAL AUTHORSHIP

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ACKNOWLEDGEMENTS

This research project would not have been possible without the support of many people. Firstly, I offer my sincerest gratitude to Professor Theresa McDonagh, whose encouragement, patience and guidance have been invaluable, not only to the development of my research skills, but to my knowledge of heart failure in all its forms and guises. To Professor Ajay Shah I extend my deepest thanks for his support and wisdom throughout this process.

Special thanks are owed to the Specialist Heart Failure Nurses of King's College Hospital, whose dedication to patient care and assistance in recruitment were second to none, and to Professor Roy Sherwood and Ms Tracy Dew of the research laboratory at King's College Hospital, for their biochemical expertise and advice.

I wish to convey my gratitude to both the British Heart Foundation and the London (South) Comprehensive Local Research Network (CLRN) for their assistance in funding this project, and the National Institute for Health Research/Wellcome Trust Clinical Research Facility based at King's College London for the use of their facilities.

Finally, I would like to thank my family and friends, who have allowed me to use them as sounding boards, proof readers and social secretaries – names are not required but this could not have been achieved without them.

'Somewhere, something incredible is waiting to be known'

Carl Sagan

ABSTRACT

Heart failure is a serious, life-threatening condition. Despite significant advances in both pharmacological and device therapies, significant morbidity and mortality remain a reality. The ability to monitor, identify and intervene on those most at risk to prevent further clinical deterioration continues to be a goal of healthcare providers around the world.

Despite their established role in the diagnosis and prognosis of heart failure, use of the B-type natriuretic peptides, BNP and NTproBNP to monitor patients with chronic heart failure has been disappointing. Such results have, in part, been attributed to the high biological variability of these peptides. Over the past decade numerous novel biomarkers associated with heart failure have been identified. Several of these have shown significant prognostic potential. Evidence supporting their use for monitoring remains lacking.

The aim of this thesis is to assess the potential of four novel biomarkers of heart failure for monitoring pharmacologically optimised, stable chronic heart failure patients, both in terms of their biological variability and how this translates into the ability for changes in serial measurements to predict cardiovascular admission. Mid-regional pro-adrenomedullin, apelin, soluble ST2 and galectin-3 were chosen due to previous promising results with respect to prognosis and their range of reflection of several different underlying pathological processes.

The hypothesis was that that one or more of these biomarkers would have lower biological variability than NTproBNP and that this would translate into serial changes being better at predicting cardiovascular admission. This being so, it would then provide strong evidence to justify a randomised controlled trial of monitoring chronic heart failure patients using one or more of these novel biomarkers.

Results demonstrated that all four novel biomarkers show lower biological variability than that of NTproBNP. This, however, did not consistently translate into improved ability for serial measurements to predict cardiovascular admission. The use of biomarkers for monitoring may depend more on the pathophysiological process being measured and the association of any marker with that process. Biomarkers of hypertrophy and fibrosis, galectin-3 and sST2, appear to show the most potential for monitoring purposes. This thesis supports the development of randomised controlled trials to assess these markers in greater detail.

CONTENTS

STATEMENT OF ORIGINAL AUTHORSHIP	2
ACKNOWLEDGEMENTS	3
ABSTRACT	4
LIST OF FIGURES	15
LIST OF TABLES	20
ABBREVIATIONS AND DEFINITIONS	23
INTRODUCTION	26
1.1 Systolic Heart Failure	27
1.1.1 Definition	27
1.1.2 Epidemiology	28
1.1.3 Aetiology	29
1.1.4 Pathophysiology	31
1.1.4.1 Normal cardiac physiology	31
1.1.4.2 Pathophysiology of left ventricular systolic dysfunction	31
1.1.5 Diagnosis	34
1.1.6 Medical therapy	35
1.1.6.1 Angiotensin Converting Enzyme Inhibitors (ACE-I)	37
1.1.6.2 Beta Blockers (β -blockers)	37
1.1.6.3 Mineralocorticoid Receptor Antagonists (MRA)	37
1.1.6.4 Angiotensin Receptor Blockers (ARB)	38
1.1.6.5 Diuretics	38
1.1.6.6 Digoxin	39
1.1.6.7 Ivabradine	39
1.1.6.8 Hydralazine and Isosorbide Dinitrate (ISDN)	40

1.1.6.9	Sacubitril/Valsartan	41
1.1.7	Device Therapy	42
1.1.7.1	Implantable Cardioverter Defibrillator (ICD)	42
1.1.7.2	Cardiac Resynchronisation Therapy (CRT)	42
1.1.8	Mechanical Support & Transplantation	44
1.1.8.1	Intra-aortic balloon pump (IABP)	44
1.1.8.2	Percutaneous/Short Term Ventricular Assist Devices (pVAD)	44
1.1.8.3	Implantable/Long Term Ventricular Assist Devices (VAD)	46
1.1.8.4	Cardiac Transplantation	47
1.1.9	Outcomes	47
1.1.10	Monitoring	48
1.1.10.1	Remote Monitoring – with implanted devices	48
1.1.10.2	Remote Monitoring – without implanted devices	49
1.1.10.3	Telephone Support	50
1.1.10.4	Biomarkers	50
1.2	Biomarkers	51
1.2.1	Definition	51
1.2.2	Clinical value of biomarkers	52
1.2.2.1	Diagnosis	52
1.2.2.1.1	Pre-test probability	53
1.2.2.1.2	Likelihood ratios	54
1.2.2.1.3	Post-test probability	55
1.2.2.2	Screening	55
1.2.2.3	Prognosis	56
1.2.2.4	Monitoring	57
1.2.3	Biological variability of biomarkers	57

1.2.4	Biomarkers of chronic heart failure	60
1.3	B-type Natriuretic Peptides	61
1.3.1	BNP and NTproBNP release and cardiovascular effects	61
1.3.2	BNP and NTproBNP for diagnosis of heart failure	63
1.3.3	BNP and NTproBNP for prognosis of heart failure	64
1.3.4	BNP and NTproBNP for monitoring heart failure	64
1.3.5	Biological variability of BNP and NTproBNP	68
1.4	Mid-Regional pro-Adrenomedullin (MRproADM)	69
1.4.1	Adrenomedullin release and cardiovascular effects	69
1.4.2	Adrenomedullin for diagnosis of heart failure	72
1.4.3	Adrenomedullin for prognosis of heart failure	72
1.4.4	Adrenomedullin for monitoring heart failure	72
1.4.5	Biological variability of adrenomedullin	73
1.5	Apelin	74
1.5.1	Apelin release and cardiovascular effects	74
1.5.2	Apelin for diagnosis of heart failure	76
1.5.3	Apelin for prognosis of heart failure	77
1.5.4	Apelin for monitoring heart failure	77
1.5.5	Biological variability of apelin	77
1.6	ST2	78
1.6.1	ST2 release and cardiovascular effects	78
1.6.2	ST2 for diagnosis of heart failure	79
1.6.3	ST2 for prognosis of heart failure	80
1.6.4	ST2 for monitoring heart failure	81
1.6.5	Biological variability of ST2	81
1.7	Galectin-3	83

1.7.1	Galectin-3 release and cardiovascular effects	83
1.7.2	Galectin-3 for diagnosis of heart failure	84
1.7.3	Galectin-3 for prognosis of heart failure	85
1.7.4	Galectin-3 for monitoring heart failure	85
1.7.5	Biological variability of galectin-3	85
RESEARCH OBJECTIVES		87
MATERIALS AND METHODS		89
3.1	Study population	90
3.2	New York Heart Association (NYHA) classification	91
3.3	12 lead ECG	91
3.4	Echocardiography	91
3.5	Biochemical analysis	92
3.5.1	NTproBNP	92
3.5.2	Mid-Regional pro-Adrenomedullin	92
3.5.3	Apelin	93
3.5.4	Soluble ST2	93
3.5.5	Galectin-3	93
3.5.6	Creatinine	94
3.5.7	Estimated Glomerular Filtration Rate (eGFR)	94
3.5.8	Sodium (Na)	94
3.5.9	Haemoglobin (Hb)	95
3.5.10	Bilirubin	96
3.6	Statistical analysis	96
3.6.1	Sample Size/Power Calculation	96
3.6.2	Descriptive Statistics	96
3.6.3	Inferential Statistics	97

3.7	Biological variability	98
3.7.1	Total Coefficient of Variation (CV_t)	98
3.7.2	Analytical Coefficient of Variation (CV_a)	99
3.7.3	Intra-individual Coefficient of Variation (CV_i)	99
3.7.4	Inter-individual Coefficient of Variation (CV_g)	99
3.7.5	Reference Change Value (RCV)	99
3.7.6	Index of Individuality (II)	100
3.8	Ethics review and ethical conduct of the study	100
RESULTS		101
PATIENT CHARACTERISTICS		102
4.1	Baseline patient characteristics	103
MID-REGIONAL PRO-ADRENOMEDULLIN		108
5.1	Introduction	109
5.2	Methods	109
5.3	Results	109
5.3.1	Patient Characteristics	109
5.3.2	Biomarker Concentrations	109
5.4	Biological Variability	111
5.4.1	Analytical Coefficient of Variation (CV_a)	111
5.4.2	Individual Coefficients of Variation, Index of Individuality and Reference Change Values	111
5.4.3	Individual Coefficients of Variation, Index of Individuality and Reference Change Values: Stable Patients	112
5.5	Serial Monitoring	113
5.5.1	Baseline correlations	113
5.5.2	Single absolute concentrations and cardiovascular events	113

5.5.3	Performance characteristics of serial MRproADM as a discriminator of patient risk	114
5.5.4	MRproADM concentrations and renal function	118
5.6	Discussion	119
5.7	Conclusion	121
APELIN		122
6.1	Introduction	123
6.2	Methods	123
6.3	Results	123
6.3.1	Patient Characteristics	123
6.3.2	Biomarker Concentrations	123
6.4	Biological Variability	125
6.4.1	Analytical Coefficient of Variation (CV_a)	125
6.4.2	Individual Coefficients of Variation, Index of Individuality and Reference Change Values	125
6.4.3	Individual Coefficients of Variation, Index of Individuality and Reference Change Values: Stable Patients	126
6.5	Serial Monitoring	127
6.5.1	Baseline correlations	127
6.5.2	Single absolute concentrations and cardiovascular events	127
6.5.3	Performance characteristics of serial apelin as a discriminator of patient risk	128
6.5.4	Apelin concentrations and renal function	132
6.6	Discussion	132
6.7	Conclusion	134
SOLUBLE ST2 (sST2)		135

7.1	Introduction	136
7.2	Methods	136
7.3	Results	136
7.3.1	Patient Characteristics	136
7.3.2	Biomarker Concentrations	137
7.4	Biological Variability	138
7.4.1	Analytical Coefficient of Variation (CV _a)	138
7.4.2	Individual Coefficients of Variation, Index of Individuality and Reference Change Values	138
7.4.3	Individual Coefficients of Variation, Index of Individuality and Reference Change Values: Stable Patients	139
7.5	Serial Monitoring	140
7.5.1	Baseline correlations	140
7.5.2	Single absolute concentrations and cardiovascular events	141
7.5.3	Performance characteristics of serial sST2 as a discriminator of patient risk	141
7.5.4	sST2 concentrations and renal function	145
7.6	Discussion	146
7.7	Conclusion	148
GALECTIN-3		149
8.1	Introduction	150
8.2	Methods	150
8.3	Results	151
8.3.1	Patient Characteristics	151
8.3.2	Biomarker Concentrations	151
8.4	Biological Variability	152

8.4.1	Analytical Coefficient of Variation (CV_a)	152
8.4.2	Individual Coefficients of Variation, Index of Individuality and Reference Change Values	152
8.4.3	Individual Coefficients of Variation, Index of Individuality and Reference Change Values: Stable Patients	153
8.5	Serial Monitoring	154
8.5.1	Baseline correlations	154
8.5.2	Single absolute concentrations and cardiovascular events	154
8.5.3	Performance characteristics of serial Galectin-3 as a discriminator of patient risk	155
8.5.4	Galectin-3 concentrations and renal function	159
8.6	Discussion	159
8.7	Conclusion	161
MULTI-MARKER ANALYSIS		162
9.1	Introduction	163
9.2	Methods	164
9.3	Results	164
9.3.1	Patient Characteristics	164
9.3.2	Baseline correlations	164
9.3.3	Identification of candidate biomarkers	165
9.3.4	Multi-marker analysis	167
9.4	Discussion	170
9.5	Conclusion	171
LIMITATIONS OF THE STUDY		172
10.1	Sample size	173
10.2	Study design	173

10.2.1	Study population	173
10.2.2	Study endpoint	174
10.2.3	Follow-up period	175
10.3	Single samples	175
10.4	Biomarker assays	176
10.4.1	MRproADM	177
10.4.2	Apelin	177
10.4.3	sST2	178
10.4.4	Galectin-3	178
DISCUSSION		180
PUBLICATIONS ARISING FROM THIS THESIS		186
	Original Articles	187
	Abstracts	188
OTHER PUBLICATIONS		190
	Review Articles	191
	Abstracts	191
REFERENCES		193

LIST OF FIGURES

Figure 1:	Comparison of cardiac systolic and diastolic dysfunction	27
Figure 2:	Epidemiology of symptomatic heart failure	28
Figure 3:	Age and gender demographics of UK heart failure admissions 2014-15	29
Figure 4:	Haemodynamic parameters involved in determining cardiac output	31
Figure 5:	Renin-Angiotensin-Aldosterone (RAAS) axis in heart failure	32
Figure 6:	Actions of the sympathetic nervous system in heart failure	32
Figure 7:	Neurohormonal and compensatory mechanisms in heart failure	33
Figure 8:	2012 ESC guidelines for the diagnosis of suspected heart failure	35
Figure 9:	2012 ESC guidelines for the management of left ventricular systolic dysfunction	36
Figure 10:	Influence of biomarker measurement on health outcomes	52
Figure 11:	Fagan nomogram showing post-test probabilities for a positive and negative diagnostic test result	55
Figure 12:	Enzymatic cleavage of proBNP into biologically active BNP and the inactive NTproBNP fragment	61
Figure 13:	Actions of BNP	62
Figure 14:	Odds ratio for all-cause mortality in monitoring trials	67
Figure 15:	Odds ratio of all-cause hospitalisation in monitoring trials	67
Figure 16:	Actions of adrenomedullin in acute heart failure	71
Figure 17:	Proposed role of apelin in heart failure	75
Figure 18:	Proposed actions of apelin in cardiac myocyte contraction	77
Figure 19:	ROC analysis of single absolute NTproBNP concentrations and CV admission	114
Figure 20:	ROC analysis of single absolute MRproADM concentrations and CV admission	114

Figure 21:	Box-plot analysis of % change in MRproADM over six months according to CV admission status	115
Figure 22:	Box-plot analysis of % change in NTproBNP over six months according to CV admission status	115
Figure 23:	ROC analysis of absolute change in MRproADM and NTproBNP over one month and CV admission	116
Figure 24:	ROC analysis of absolute change in MRproADM and NT proBNP over three months and CV admission	116
Figure 25:	ROC analysis of absolute change in MRproADM and NTproBNP over six months and CV admission	116
Figure 26:	ROC analysis of % change in MRproADM and NTproBNP over one month and CV admission	117
Figure 27:	ROC analysis of % change in MRproADM and NTproBNP over three months and CV admission	117
Figure 28:	ROC analysis of % change in MRproADM and NTproBNP over six months and CV admission	117
Figure 29:	ROC analysis of % change in MRproADM and NTproBNP over six months and worsening renal function	118
Figure 30:	ROC analysis of single absolute NTproBNP concentrations and CV admission	128
Figure 31:	ROC analysis of single absolute apelin concentrations and CV admission	128
Figure 32:	Box-plot analysis of % change in apelin over 6 months according to CV admission status	129
Figure 33:	Box-plot analysis of % change in NTproBNP over 6 months according to CV admission status	129
Figure 34:	ROC analysis of absolute change in apelin and NTproBNP over one month and CV admission	130

Figure 35:	ROC analysis of absolute change in apelin and NTproBNP over three months and CV admission	130
Figure 36:	ROC analysis of absolute change in apelin and NTproBNP over six months and CV admission	130
Figure 37:	ROC analysis of % change in apelin and NTproBNP over one month and CV admission	131
Figure 38:	ROC analysis of % change in apelin and NTproBNP over three months and CV admission	131
Figure 39:	ROC analysis of % change in apelin and NTproBNP over six months and CV admission	131
Figure 40:	ROC analysis of single absolute NTproBNP concentrations and CV admission	141
Figure 41:	ROC analysis of single absolute sST2 concentrations and CV admission	141
Figure 42:	Box-plot analysis of % change in sST2 over 6 months according to CV admission status	142
Figure 43:	Box-plot analysis of % change in NTproBNP over 6 months according to CV admission status	142
Figure 44:	ROC analysis of absolute change in sST2 and NTproBNP over one month and CV admission	143
Figure 45:	ROC analysis of absolute change in sST2 and NTproBNP over three months and CV admission	143
Figure 46:	ROC analysis of absolute change in sST2 and NTproBNP over six months and CV admission	143
Figure 47:	ROC analysis of % change in sST2 and NTproBNP over one month and CV admission	144

Figure 48:	ROC analysis of % change in sST2 and NTproBNP over three months and CV admission	144
Figure 49:	ROC analysis of % change in sST2 and NTproBNP over six months and CV admission	144
Figure 50:	ROC analysis of absolute change in sST2 and NTproBNP over six months and worsening renal function	145
Figure 51:	ROC analysis of % change in sST2 and NTproBNP over six months and worsening renal function	145
Figure 52:	ROC analysis of single absolute NTproBNP concentrations and CV admission	155
Figure 53:	ROC analysis of single absolute galectin-3 concentrations and CV admission	155
Figure 54:	Box-plot analysis of % change in galectin-3 over 6 months according to CV admission status	156
Figure 55:	Box-plot analysis of % change in NTproBNP over 6 months according to CV admission status	156
Figure 56:	ROC analysis of absolute change in galectin-3 and NTproBNP over one month and CV admission	157
Figure 57:	ROC analysis of absolute change in galectin-3 and NTproBNP over three months and CV admission	157
Figure 58:	ROC analysis of absolute change in galectin-3 and NTproBNP over six months and CV admission	157
Figure 59:	ROC analysis of % change in galectin-3 and NTproBNP over one month and CV admission	158
Figure 60:	ROC analysis of % change in galectin-3 and NTproBNP over three months and CV admission	158
Figure 61:	ROC analysis of % change in galectin-3 and NTproBNP over six months	158

and CV admission

Figure 62: ROC analysis of % change of all studied biomarkers over six months **166**

and CV admission

Figure 63: ROC analysis of % change in clinical biomarkers over six months and CV **166**

admission

Figure 64: CV admission according to number of biomarkers with % change above **167**

the median

Figure 65: CV admission according to % change in sST2 and/or galectin-3 above the **169**

median

LIST OF TABLES

Table 1:	Different aetiologies of heart failure	30
Table 2:	Comparison of IABP and pVADs for cardiogenic shock	45
Table 3:	Clinical trials of pVADs vs. IABP support	45
Table 4:	Factors affecting and steps to reduce variability	58
Table 5:	Cardiac biomarkers of myocardial insult, neurohormonal activation and remodelling	60
Table 6:	Summary of trials examining the use of the B-Type Natriuretic Peptides for monitoring chronic heart failure	66
Table 7:	Reference concentrations for MRproADM in normal subjects	93
Table 8:	Baseline patient characteristics	103
Table 9:	Baseline mean prognostic medication doses	104
Table 10:	Admission details for the eight patients with a CV admission	105
Table 11:	Baseline demographics of those with and without a CV admission	106
Table 12:	Median concentrations of NTproBNP and MRproADM at each time point	110
Table 13:	CV _g , CV _i , index of individuality and corresponding reference change values for NTproBNP and MRproADM at each time point	111
Table 14:	CV _g , CV _i , index of individuality and corresponding reference change values for NTproBNP and MRproADM at each time point for patients not experiencing a CV admission	112
Table 15:	Pearson correlation of baseline NTproBNP and MRproADM with patient characteristics related to prognosis	113
Table 16:	Comparison of median interquartile range (IQR) percentage changes for NTproBNP and MRproADM overall and for those with and without a CV admission	115

Table 17:	Median concentrations of NTproBNP and apelin at each time point	124
Table 18:	CV _g , CV _i , index of individuality and corresponding reference change values for NTproBNP and apelin at each time point	125
Table 19:	CV _g , CV _i , index of individuality and corresponding reference change values for NTproBNP and apelin at each time point for patients not experiencing a CV admission	126
Table 20:	Pearson correlation of baseline NTproBNP and apelin with patient characteristics related to prognosis	127
Table 21:	Comparison of median interquartile range (IQR) percentage changes for NTproBNP and apelin overall and for those with and without a CV admission	129
Table 22:	Median concentrations of NTproBNP and sST2 at each time point	137
Table 23:	CV _g , CV _i , index of individuality and corresponding reference change values for NTproBNP and sST2 at each time point.	138
Table 24:	CV _g , CV _i , index of individuality and corresponding reference change values for NTproBNP and sST2 at each time point for patients not experiencing a CV admission	139
Table 25:	Pearson correlation of baseline NTproBNP and sST2 with patient characteristics related to prognosis	140
Table 26:	Comparison of median interquartile range (IQR) percentage changes for NTproBNP and sST2 overall and for those with and without a CV admission	142
Table 27:	Median concentrations of NTproBNP and galectin-3 at each time point	151
Table 28:	CV _g , CV _i , index of individuality and corresponding reference change values for NTproBNP and galectin-3 at each time point	152
Table 29:	CV _g , CV _i , index of individuality and corresponding reference change values for NTproBNP and galectin-3 at each time point for patients not experiencing a CV admission	153

Table 30:	Pearson correlation of baseline NTproBNP and galectin-3 with patient characteristics related to prognosis	154
Table 31:	Comparison of median interquartile range (IQR) percentage changes for NTproBNP and galectin-3 overall and for those with and without a CV admission	156
Table 32:	Baseline biomarker correlations	165
Table 33:	Change in biomarkers above or below the median % change over six months in patients with a CV admission	168
Table 34:	Change in biomarkers above or below the median % change over six months in patients with a CV admission relative to time of admission	170
Table 35:	Percentage CV_a of CV_i for NTproBNP, MRproADM, apelin, sST2 and galectin-3	175
Table 36:	Percentage CV_a of CV_i for NTproBNP, MRproADM, apelin, sST2 and galectin-3 for patients not experiencing a CV admission	176

ABBREVIATIONS AND DEFINITIONS

ACE-I	Angiotensin converting enzyme inhibitor
ADM	Adrenomedullin
AHF	Acute heart failure
Ang II	Angiotensin II
ANP	Atrial natriuretic peptide
APJ	Apelin receptor
ARB	Angiotensin II receptor blocker
AUC	Area under curve
β-blocker	Beta-blocker
BNP	Brain natriuretic peptide
cAMP	Cyclic adenosine monophosphate
CHF	Chronic heart failure
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin gene related peptide
CNP	C-type natriuretic peptide
CRP	C-reactive protein
CRT	Cardiac resynchronisation therapy
CV	Cardiovascular
CV_a	Analytical coefficient of variation
CV_g	Inter-individual coefficient of variation
CV_i	Intra-individual coefficient of variation
CV_t	Total coefficient of variation
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate

EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ESC	European Society of Cardiology
Hb	Haemoglobin
HF	Heart failure
IL-33	Interleukin 33
IQR	Interquartile range
LBBB	Left bundle branch block
LVEF	Left ventricular ejection fraction
LVSD	Left ventricular systolic dysfunction
MD	Doctorate of medicine
MRA	Mineralocorticoid receptor antagonist
mRNA	Messenger ribonucleic acid
MRproADM	Mid-regional pro-adrenomedullin
MRproANP	Mid-regional pro-atrial natriuretic peptide
Na	Sodium
NICE	National Institute for Health and Care Excellence
NO	Nitric oxide
NPPB	Natriuretic peptide precursor B
NPR-A	Natriuretic peptide receptor A
NPV	Negative predictive value
NRES	National Research Ethics Service
NTproANP	N-terminal pro-atrial natriuretic peptide
NTproBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association

OLT	Orthotopic liver transplantation
preproADM	Pre-pro-adrenomedullin
RAAS	Renin-angiotensin-aldosterone system
RCT	Randomised controlled trial
RCV	Reference change value
RIA	Radioimmunoassay
RNA	Ribonucleic acid
ROC	Receiver operator curve
RRR	Relative risk reduction
SD	Standard deviation
ST2	Suppression of tumorigenicity 2
sST2	Soluble suppression of tumorigenicity 2
ST2L	Transmembrane suppression of tumorigenicity
STEMI	ST elevation myocardial infarction
SV	Stroke volume
TNFα	Tumour necrosis factor alpha
UK	United Kingdom

INTRODUCTION

1.1 Systolic Heart Failure

1.1.1 Definition

Described by Harrison's group as 'primary disorders of filling and primary disorders of emptying'⁽¹⁾, heart failure (HF) is the finishing line of numerous cardiovascular (CV) disorders. It occurs as a result of an impaired ability of the heart to function as a pump to support physiological circulation. Conceptually, it is a syndrome rather than a definitive diagnosis, typified by symptoms of breathlessness and fatigue combined with signs of fluid overload.

The clinical syndrome of HF may result from disorders of the pericardium, myocardium, endocardium, great vessels, or conducting system but the majority of patients have symptoms due to left ventricular myocardial dysfunction. As alluded to by Harrison⁽¹⁾, such dysfunction may manifest as disorders of diastole (filling), systole (emptying) (figure 1), or a combination of the two.

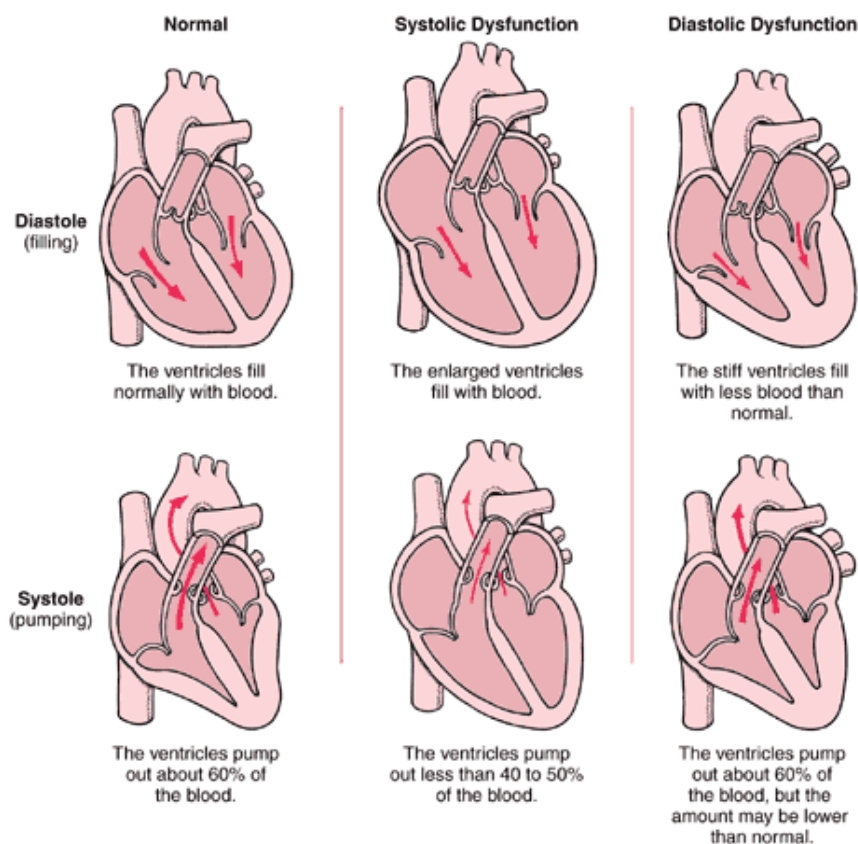


Figure 1: Comparison of cardiac systolic and diastolic dysfunction⁽²⁾

Whilst much attention has been borne to the understanding of both of these manifestations, it is the pathophysiology, epidemiology, diagnosis and treatment of chronic HF resulting from left ventricular systolic dysfunction (LVSD) that has been at the forefront of clinical advancements over the past three decades⁽³⁻⁸⁾.

1.1.2 Epidemiology

The epidemiology of symptomatic HF has been well characterised in developing countries (figure 2), and, in particular, in Europe⁽⁹⁻¹⁶⁾.

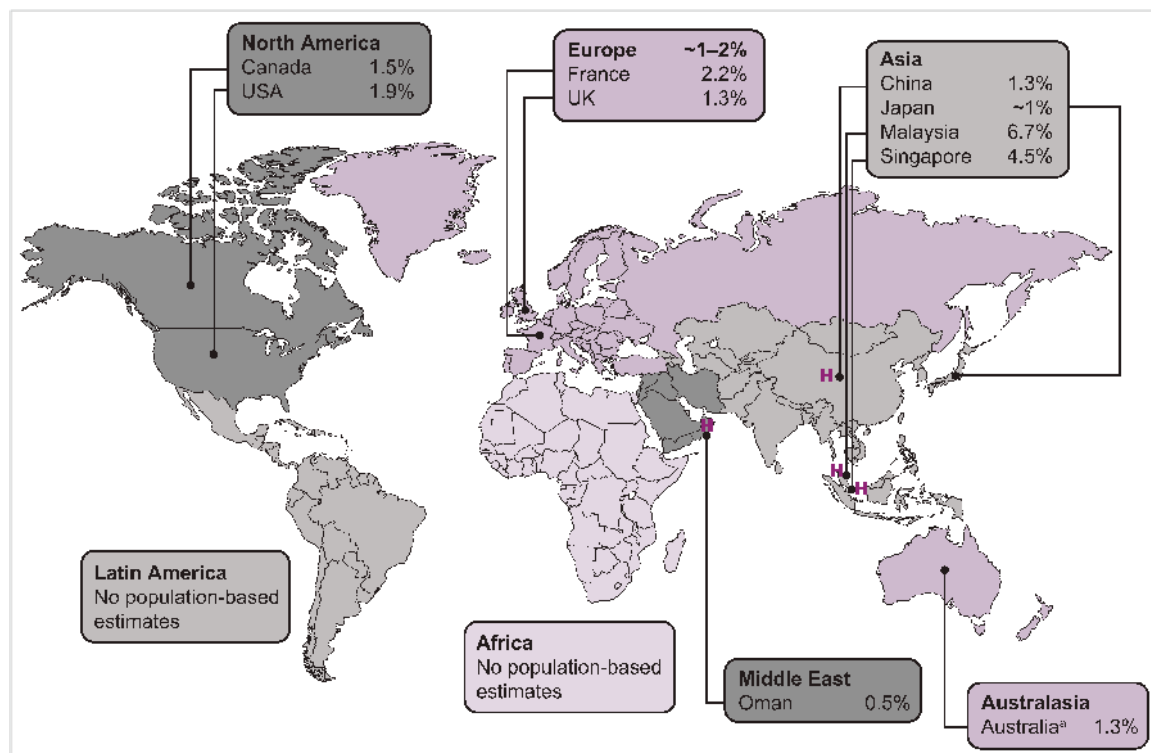


Figure 2: Epidemiology of symptomatic heart failure⁽¹⁷⁾

In these settings, the overall prevalence is estimated at approximately 1-2% of the adult population but is markedly higher in the elderly; with as many as 15% of those aged over 85 years affected⁽¹⁸⁾. Previous UK studies have demonstrated an annual incidence of 0.12% in those aged 55-64, rising to 1.2% in those aged over 85. This translates into an estimated 63,000 new cases in the UK each year⁽¹⁹⁾. Incidence is higher in men than in women in all age groups up to the age of 85 years (figure 3)⁽²⁰⁾.

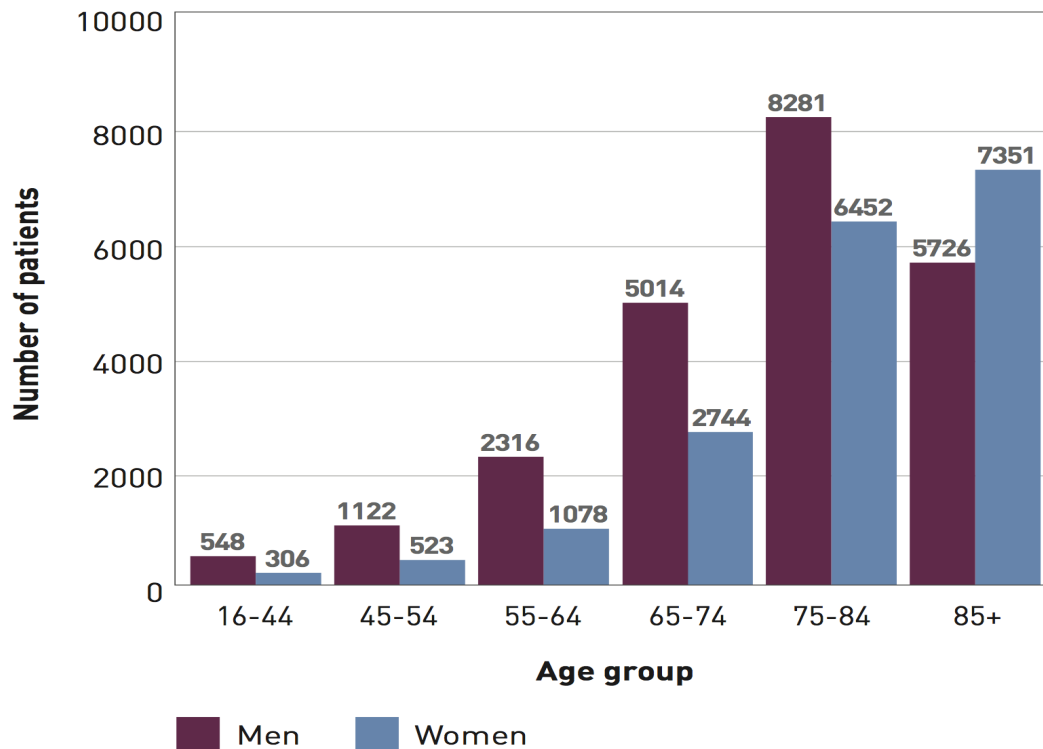


Figure 3: Age and gender demographics of UK heart failure admissions 2014-15⁽²⁰⁾

From a health-economic perspective, it is one of the most common reasons for emergency admission, readmission and bed occupancy. Recent estimates suggest HF accounts for one million NHS inpatient bed-days, equating to 2% of the NHS total and 5% of all emergency hospital admissions⁽²¹⁾.

1.1.3 Aetiology

Throughout the Western world, coronary artery disease (CAD), either alone or in combination with hypertension, is accountable for approximately two-thirds of cases of LVSD. In a study of left ventricular function in North Glasgow⁽⁹⁾, 95% vs 71% of symptomatic and asymptomatic individuals with definite LVSD had evidence of CAD ($p = 0.04$). Those individuals with symptoms were also more likely to have a history of myocardial infarction (50% vs 14%; $p = 0.01$) and concurrent angina (62% vs 43%; $p = 0.02$). Furthermore, hypertension (80%) and valvular heart disease (25%) were also more prevalent in those individuals with both clinical and echocardiographically determined HF compared to the remainder of the cohort (67% and 0%, respectively).

Despite such findings, there are many other identifiable causes of HF (table 1), the prevalence of which will depend on the geographical location and demographics of the cohort examined. In the ageing population aetiological certainty is challenging with patients presenting with multiple potential causes (for example, CAD, hypertension, diabetes mellitus, atrial fibrillation, etc.).

Aetiology of Heart Failure	
There is no agreed or satisfactory classification for the causes of HF, with much overlap between potential categories	
Myocardial disease	
1. Coronary artery disease	
2. Hypertension ^a	
3. Cardiomyopathy ^b	
a. Familial	
i. Hypertrophic	
ii. Dilated	
iii. Arrhythmogenic right ventricular cardiomyopathy	
iv. Restrictive	
v. Left ventricular non-compaction	
b. Acquired ^c	
i. Myocarditis (inflammatory cardiomyopathy)	
Infective	
• Bacterial	
• Spirochaetal	
• Fungal	
• Protozoal	
• Parasitic	
• Rickettsial	
• Viral	
Immune-mediated	
• Tetanus toxoid, vaccines, serum sickness	
• Drugs	
• Lymphocytic/giant cell myocarditis	
• Sarcoidosis	
• Autoimmune	
• Eosinophilic (Churg–Strauss)	
Toxic	
• Drugs (e.g. chemotherapy, cocaine)	
• Alcohol	
• Heavy metals (copper, iron, lead)	
ii. Endocrine/nutritional	
• Pheochromocytoma	
• Vitamin deficiency (e.g. thiamine)	
• Selenium deficiency	
• Hypophosphataemia	
• Hypocalcaemia	
iii. Pregnancy	
iv. Infiltration	
• Amyloidosis	
• Malignancy	
Valvular heart disease	
Mitral	
Aortic	
Tricuspid	
Pulmonary	
Pericardial disease	
Constrictive pericarditis	
Pericardial effusion	
Endocardial disease	
• Endomyocardial diseases with hypereosinophilia [hypereosinophilic syndromes (HES)]	
• Endomyocardial disease without hypereosinophilia [e.g. endomyocardial fibrosis (EMF)]	
• Endocardial fibroelastosis	
Congenital heart disease	
Arrhythmia	
Tachyarrhythmia	
Atrial	
Ventricular	
Bradyarrhythmia	
Sinus node dysfunction	
Conduction disorders	
• Atrioventricular block	
High output states	
• Anaemia	
• Sepsis	
• Thyrotoxicosis	
• Paget's disease	
• Arteriovenous fistula	
Volume overload	
• Renal failure	
• Iatrogenic (e.g. post-operative fluid infusion)	

Table 1: Different aetiologies of heart failure⁽²²⁾

1.1.4 Pathophysiology

1.1.4.1 Normal cardiac physiology

The amount of blood pumped by the heart over a given time period is known as cardiac output, which is in turn the product of heart rate and stroke volume (SV) and, in the normal heart, is typically 4–8 L/min^(23, 24). In addition, other factors such as synergistic ventricular contraction, ventricular wall integrity, and valvular competence all affect cardiac output (figure 4).

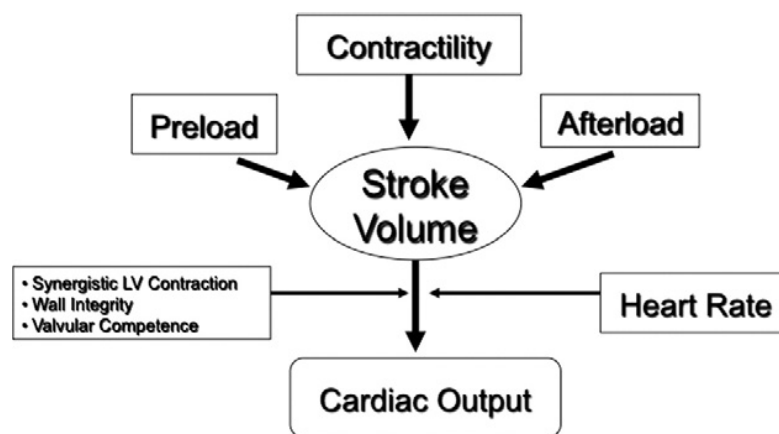


Figure 4: Haemodynamic parameters involved in determining cardiac output

SV is defined as the amount of blood ejected by the ventricle per heartbeat, and is usually 1 cc/kg or approximately 60–100ml^(23, 24). SV is affected by three main factors: preload, the amount of myocardial fibre stretch at the end of diastole; afterload, the resistance that must be overcome for the ventricle to eject blood; and contractility, the inotropic state of the heart independent of the preload or the afterload.

1.1.4.2 Pathophysiology of left ventricular systolic dysfunction

Although extensively studied, knowledge of the pathophysiology of HF remains limited. Much of our understanding is derived from studies of myocardial infarction⁽²⁵⁻²⁸⁾ and therefore, it is the mechanism of LVSD that remains the best documented. In such cases, systolic dysfunction is the result of reduced myocardial contractility or significantly increased impedance to left ventricular ejection leading to a reduction in the ability of the myocardial sarcomeres to shorten. As

physiological circulation succumbs to the resultant reduction in cardiac output and increase in wall stress, activation of complex compensatory mechanisms from the subcellular to the organ-to-organ level ensues. Many of these mechanisms rely on the activation of neurohormonal systems to maintain arterial pressure, myocyte contractility and ultimately the perfusion of vital organs. These mechanisms predominantly, but not exclusively, involve the renin-angiotensin-aldosterone system (RAAS) (figure 5) and sympathetic nervous systems (figure 6), leading to increased heart rate, vasoconstriction and salt and water retention. The subsequent expansion of blood volume is thought to utilise the principles of the Frank-Starling mechanism, whereby increased stretch of the myocyte causes increased force of contraction, thereby allowing conservation of stroke volume.

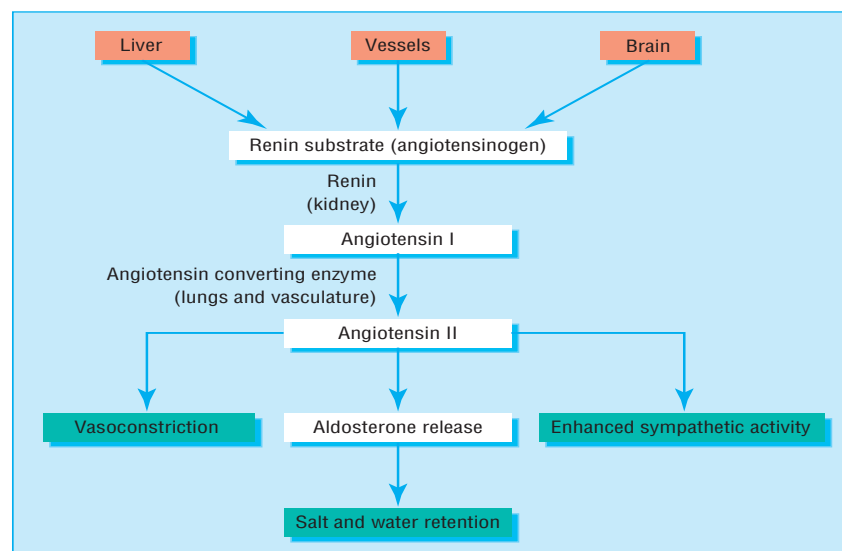


Figure 5: Renin-Angiotensin-Aldosterone (RAAS) axis in heart failure⁽²⁹⁾

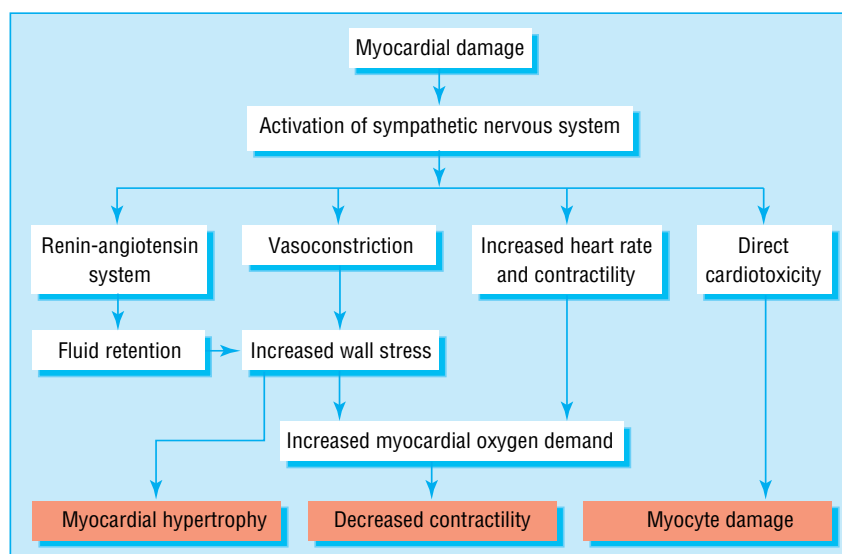


Figure 6: Actions of the sympathetic nervous system in heart failure⁽²⁹⁾

Unfortunately, as myocardial dysfunction advances, these initial compensatory mechanisms ultimately become maladaptive, resulting in increased preload (increased extracellular fluid volume and venous return), afterload (systemic arterial constriction) and myocardial energy expenditure. In an attempt to maintain left ventricular systolic wall stress within normal limits, there is increased cell proliferation and myocyte hypertrophy. Pressure overload tends towards the laying down of new sarcomeres that increase myocyte width (concentric hypertrophy), whereas volume overload leads to the replication of sarcomeres in series and elongation of myocytes (eccentric hypertrophy)^(27, 30). If left unchecked, these processes result in structural changes, leading to a reduction in fibre shortening, a decrease in left ventricular ejection fraction (LVEF) and ultimately left ventricular spherical dilatation - a phenomenon known as remodelling. Furthermore, ventricular dilatation may subsequently lead to stretching of the mitral valve ring, resulting in valvular incompetence. Although this phenomenon results in ventricular offloading via the atria, the increased workload required to maintain the oxygen demands of the body ultimately leads to worsening of ventricular function, increased arrhythmic risk and pulmonary hypertension (figure 7).

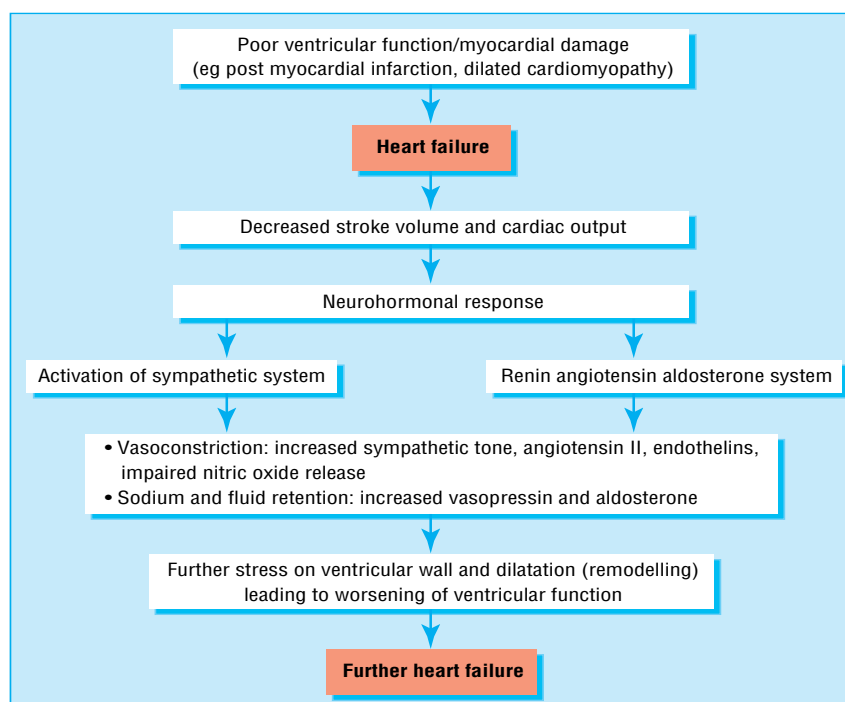


Figure 7: Neurohormonal and compensatory mechanisms in heart failure⁽²⁹⁾

These systemic processes also have detrimental effects on the functioning of the lungs, blood

vessels, kidneys, and muscles. Coupled with electrolyte imbalances, the release of inflammatory cytokines, oxygen free radicals and endothelins, the result is a pathophysiological viscous cycle, in which further electrical and mechanical dysfunction of the heart ensues.

Exceptions to these processes are the release of the natriuretic peptides and endogenous vasodilatory and inotropic peptides all of which are released in an attempt to counteract the detrimental effects of this viscous cycle and are the focus of much of the work on biomarkers for HF.

1.1.5 Diagnosis

Traditionally, the diagnosis of HF was based on clinical findings alone, particularly in the community and primary healthcare setting. The elderly and those with multiple co-morbidities, however, have always posed a challenge to the diagnostic abilities of the physician, with further advice regarding those who should then be referred for specialist opinion also being previously lacking. Indeed, some early studies suggested that less than half of those patients with a primary care setting diagnosis of HF went on to have their diagnosis confirmed with cardiac imaging and evaluation in a secondary centre⁽³¹⁾. Furthermore, given the low diagnostic yield of echocardiography in patients with only a clinical suspicion of HF, together with previously limited access to this imaging modality, many patients in this setting remained treated, but without an official diagnosis.

As a result, even since the mid-1990's, it has been recognised by both the European and American societies that a diagnosis of HF based on clinical signs alone is not possible and that this can only be accurately established via the use of cardiac imaging, most commonly echocardiography⁽³²⁻³⁶⁾. Accordingly, for the last two decades, much interest has been directed at looking to the use of biomarkers to aid diagnostic accuracy and identify those in need of further investigation. Indeed, the introduction of objective, non-invasive, biologically meaningful biomarkers to clinical assessment has considerably changed the way HF is evaluated^(21, 22) (figure 8).

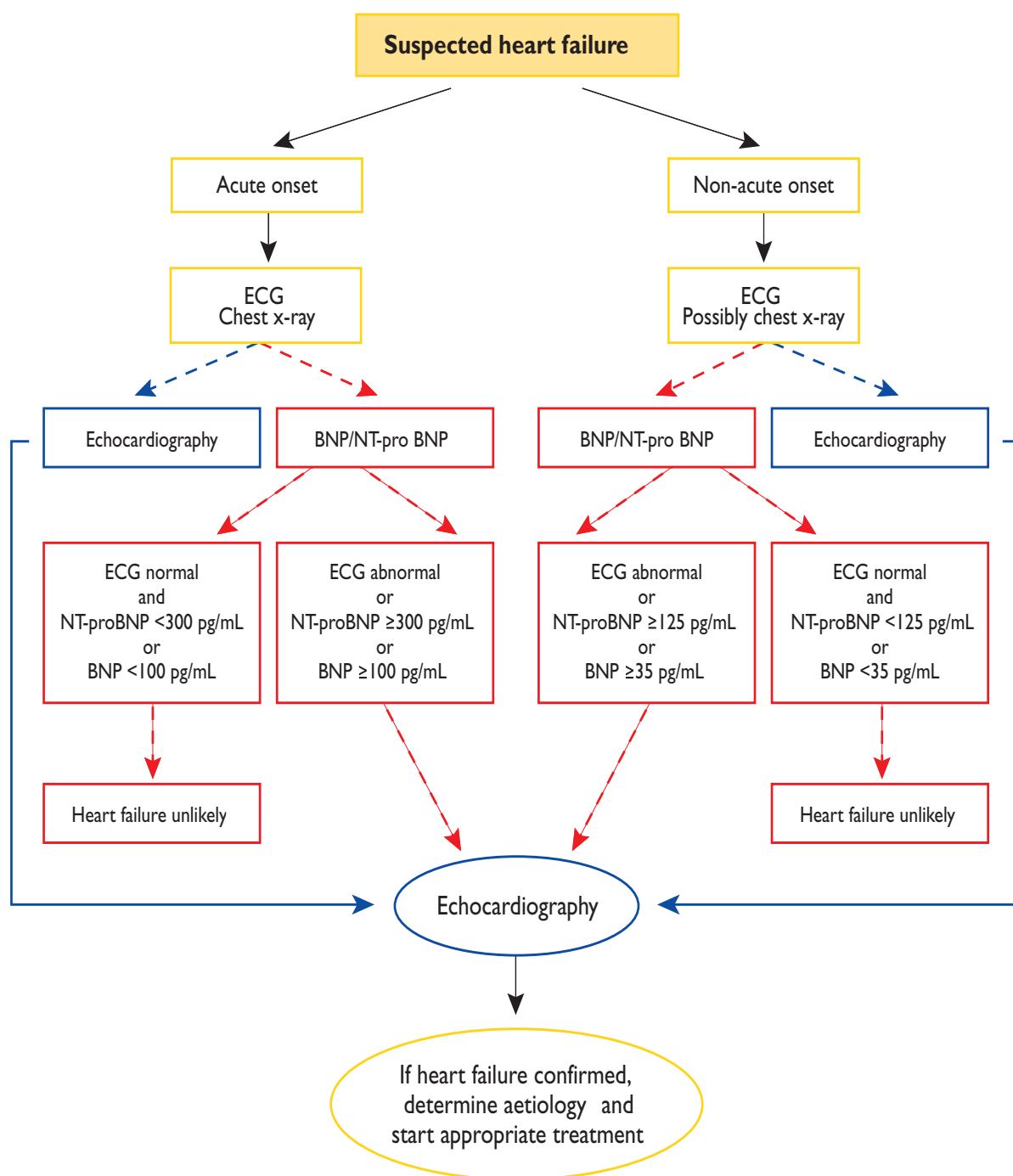


Figure 8: 2012 European Society of Cardiology (ESC) guidelines for the diagnosis of suspected heart failure⁽²²⁾

1.1.6 Medical therapy

Regardless of the underlying aetiology, treatment with prognostically indicated medications are advocated in almost all instances of HF secondary to LVSD ($EF \leq 40\%$) (figure 9)⁽²²⁾. In addition, many patients require treatment to control symptoms and reduce hospitalisations, most commonly in the form of diuretic therapy.

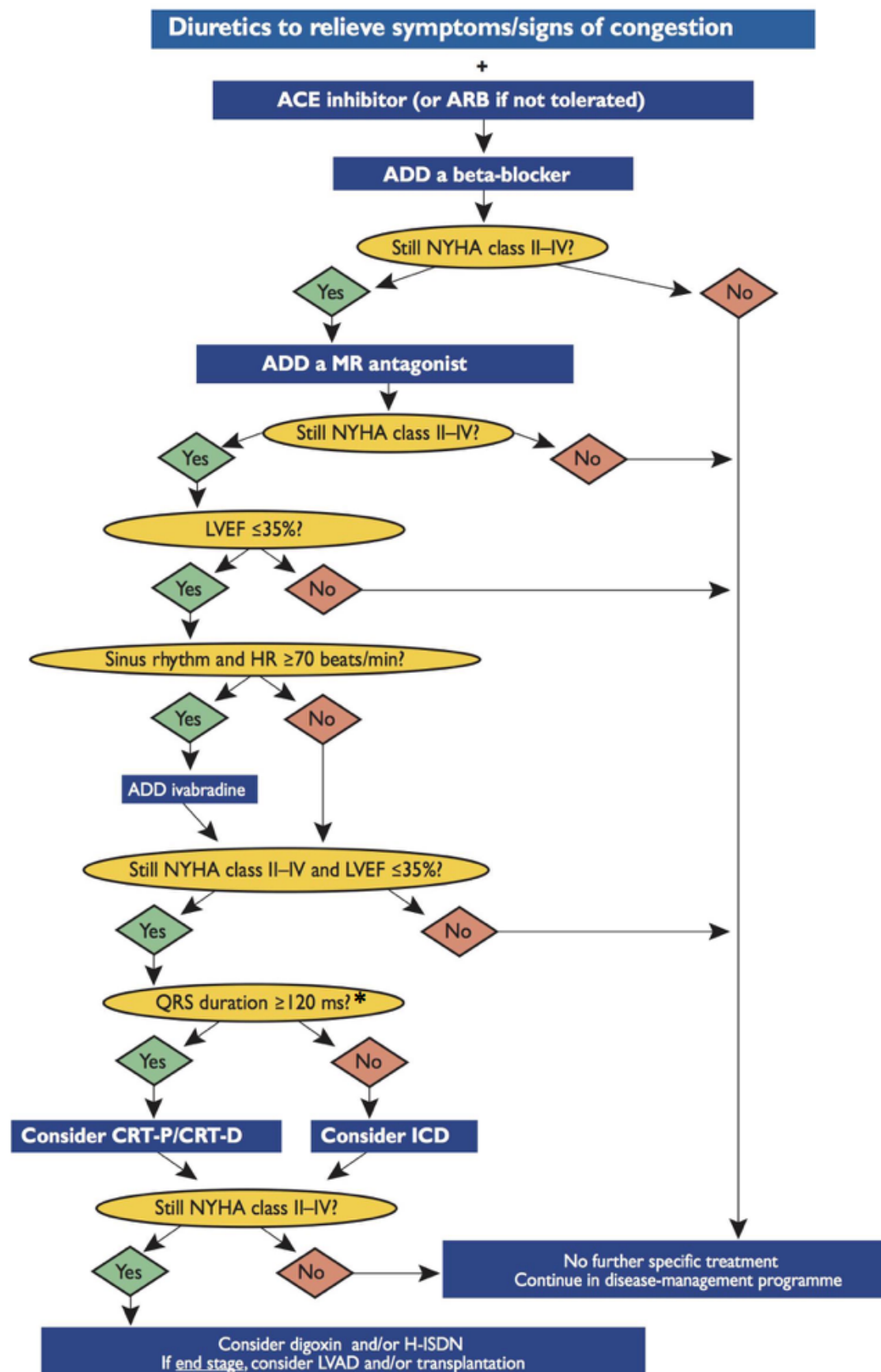


Figure 9: 2012 ESC guidelines for the management of left ventricular systolic dysfunction⁽²²⁾

*The QRS duration required for the consideration of CRT was subsequently amended to ≥ 130 ms after the results of the EchoCRT trial in 2013⁽³⁷⁾

1.1.6.1 Angiotensin Converting Enzyme Inhibitors (ACE-I)

Two key randomised controlled trials, Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS)⁽³⁾ and Studies of Left Ventricular Dysfunction (SOLVD)⁽⁴⁾, advocated the use of ACE-I in the full spectrum of patients with symptomatic HF secondary to LVSD. Results of these trials demonstrated a relative risk reduction (RRR) in mortality of 27% and 16% and an absolute risk reduction of 14.6% and 4.5% respectively. With an additional 26% relative risk reduction in HF hospitalisation observed in SOLVD and further corroboration of results by a wealth of additional studies and subsequent meta-analyses⁽³⁸⁻⁴²⁾, the 2012 European Society of Cardiology (ESC) guidelines⁽²²⁾ suggest that ACE-I are offered to all patients with LVSD (EF≤40%), with a IA level of recommendation.

1.1.6.2 Beta Blockers (β -blocker)

Despite initial fears regarding the use of beta-blockers in patients with LVSD⁽⁴³⁻⁴⁵⁾, several pivotal trials were successfully able to demonstrate, not only their safety when initiated in the chronic phase, but substantial benefits in both mortality and reverse remodelling. Three such trials, Cardiac Insufficiency Bisoprolol Study II (CIBIS II)⁽⁵⁾, Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS)⁽⁴⁶⁾, and Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF)⁽⁴⁷⁾ showed that beta-blocker treatment resulted in a RRR in mortality of approximately 34% and in HF hospitalisation of 28–36% within 1 year of starting treatment. These benefits were additional to those gained with conventional treatment, including an ACE inhibitor and have been replicated in numerous studies⁽⁴⁸⁻⁵²⁾. As with ACE-I, the 2012 ESC guidance gives a IA level of recommendation for the use of beta-blockers in all patients with LVSD (EF≤40%)⁽²²⁾.

1.1.6.3 Mineralocorticoid Receptor Antagonists (MRA)

Two key trials, Randomized Aldactone Evaluation Study (RALES)⁽⁵³⁾ and Eplerenone in Patients with Systolic Heart Failure and Mild Symptoms (EMPHASIS-HF)⁽⁵⁴⁾ have examined the use of

mineralocorticoid receptor antagonists (MRAs) in patients with EF≤35% and New York Heart Association (NYHA) functional class II-IV. Both trials demonstrated RRR in mortality and HF hospitalisations of 30-37% within approximately 2 years of starting treatment, over and above the benefits obtained from conventional therapy, including both ACE-I and beta-blockers. As a result of the EMPHASIS-HF trial, the recommendations for the use of MRAs in LVSD has increased from IB to IA in the 2012 ESC guidance, and are recommended for all patients, in addition to ACE-I and beta-blockers, with EF≤35% and NYHA class II-IV symptoms⁽²²⁾.

1.1.6.4 Angiotensin Receptor Blockers (ARB)

Angiotensin receptor blockers have a more limited evidence base than ACE-I and have not shown superiority over ACE-I in any large robust clinical trial. There are currently no compelling indications for the routine first line use of ARBs.

The CHARM-Alternative⁽⁵⁵⁾ trial was a placebo-controlled randomised controlled trial (RCT) with Candesartan in 2028 patients, intolerant of an ACE-I with an LVEF ≤40%. Treatment with Candesartan resulted in an RRR of CV mortality or HF hospitalization of 23%. The 2012 ESC guidance would therefore recommend the use of ARBs as second line agents in patients intolerant to ACE-I⁽²²⁾.

1.1.6.5 Diuretics

There are no randomised controlled trials examining the effect of diuretic therapy on mortality or morbidity in HF patients. They are, however, extremely effective at relieving the symptoms of fluid overload and are therefore recommended for all patients with such symptoms, regardless of LVEF. The aim of such treatment is to achieve and maintain euvolaemia at the lowest possible dose. Loop diuretics constitute the mainstay of therapy in the majority of patients with LVSD, with the addition of thiazide or thiazide-like diuretics in those with resistant symptoms.

1.1.6.6 Digoxin

Only one large trial has examined the use of digoxin in patients with symptomatic LVSD ($EF \leq 45\%$) who were in sinus rhythm⁽⁵⁶⁾. In this, treatment with digoxin was associated with a RRR in HF hospitalisation of 28% within three years of starting treatment. There was, however, no demonstrable mortality benefit. The 2012 ESC guidance therefore advises that digoxin may be considered to reduce the risk of HF hospitalization in patients in sinus rhythm with an $EF \leq 45\%$ who are unable to tolerate a beta-blocker or with persisting symptoms (NYHA class II–IV) despite treatment with a beta-blocker, ACE-I or ARB and an MRA⁽²²⁾. The use of digoxin in the setting of atrial fibrillation where additional rate control is required over and above that provided by beta-blockers continues to be advocated, with IB level of evidence⁽²²⁾.

1.1.6.7 Ivabradine

Several studies have demonstrated mortality benefits related to heart rate control in chronic HF patients^(3-5, 57). Ivabradine is a novel pharmacological agent that inhibits the I_f channel of the sinus node, leading to increased time of diastolic depolarisation and resulting in selective heart rate reduction.

The Systolic Heart failure treatment with the I_f Inhibitor Ivabradine Trial (SHIFT)⁽⁵⁸⁾ is the only trial to date examining the use of Ivabradine, in addition to conventional therapy, in symptomatic patients with LVSD ($EF \leq 35\%$) and heart rate ≥ 70 bpm. Results of this study demonstrated a significant benefit from the addition of Ivabradine, with a RRR in the primary composite outcome of CV death or HF hospitalization of 18%. Of note, the reduction in CV death (or all-cause death) at this target heart rate was not significant, but the RRR in HF hospitalization was 26%. Subsequently, in a subgroup analysis of patients with heart rate ≥ 75 bpm, the benefits of Ivabradine were even more pronounced, with a RRR in the primary composite endpoint of 24%, all-cause mortality of 17%, CV death of 17% and death from HF of 39%.

As a result of SHIFT, the 2012 ESC guidance for chronic HF includes the advice that Ivabradine ‘should be considered to reduce the risk of HF hospitalization in patients in sinus rhythm with an EF $\leq 35\%$, a heart rate remaining ≥ 70 bpm and persisting symptoms (NYHA class II–IV) despite treatment with an evidence-based dose of beta-blocker (or maximum tolerated dose), ACE-I or ARB and an MRA⁽²²⁾’. The Medicines and Healthcare products Regulatory Authority (MHRA) and National Institute for Health and Care Excellence (NICE), however, took a different stance, and concluded that the benefits of the drug were only significantly demonstrable in patients with heart rates of ≥ 75 bpm. For this reason, in the UK, Ivabradine is recommended only for patients in sinus rhythm, with an EF $\leq 35\%$, heart rate ≥ 75 bpm and persisting symptoms (NYHA class II–IV) despite treatment with standard evidence-based medication⁽⁵⁹⁾.

1.1.6.8 Hydralazine and Isosorbide Dinitrate (ISDN)

Results of the V-HeFT trials⁽⁶⁰⁻⁶²⁾ performed in the 1980’s and early 1990’s were the first to suggest ethnic differences in response to HF therapy. Specifically, in V-HeFT I⁽⁶⁰⁾, the combination of isosorbide dinitrate (ISDN) and hydralazine significantly reduced mortality in Black patients, but not Caucasians. Furthermore, in V-HeFT II⁽⁶¹⁾, the ACE-I enalapril provided significant benefit compared with ISDN/Hydralazine in the Caucasian population, but not in Blacks. This led the investigators to conduct the A-HeFT trial⁽⁶³⁾, enrolling only African Americans, because previous data suggested this group might obtain the greatest benefit. In A-HeFT, 1050 African-American men and women in NYHA class III or IV were randomized to placebo or ISDN/Hydralazine, added to conventional therapy. The trial was discontinued prematurely, after a median follow-up of 10 months, because of a significant reduction in mortality (RRR 43%) and risk of HF hospitalization (RRR 33%).

Despite such findings, both the V-HeFT and A-HeFT trials were relatively small, making definitive conclusions regarding the benefits of ISDN/Hydralazine combination, particularly in the non-Black patient, difficult. Consequently the combination of ISDN/Hydralazine is only recommended for use in

patients who cannot tolerate either ACE-I or ARB, or who remain symptomatic (NYHA II-IV) despite optimal therapy with ACE-I or ARB, beta-blockers and MRA with LVEF $\leq 35\%$ or LVEF $\leq 45\%$ with a dilated LV ⁽²²⁾.

1.1.6.9 Sacubitril-Valsartan

For nearly thirty years, ACE-I have been the foundation of the treatment of HF with reduced ejection fraction.

In September 2014, the results of the PARADIGM-HF trial were published⁽⁶⁴⁾. In this landmark study, the novel angiotensin-neprilysin inhibitor sacubitril-valsartan was compared with enalapril in patients with HF and reduced ejection fraction (LVEF $\leq 35\%$). The primary endpoint was a composite of death from cardiovascular causes or hospitalisation for HF. The trial was stopped early after a median follow-up of 27 months when the boundary for an overwhelming benefit in favour of sacubitril-valsartan was crossed.

Neprilysin is a neural endopeptidase which degrades several endogenous vasoactive peptides, including the natriuretic peptides, which are discussed in more detail in section 1.3 of this thesis. Inhibition of neprilysin increases the levels of these substances, counteracting the activation of pathophysiological neurohormonal processes responsible for vasoconstriction, sodium retention and ventricular remodelling⁽⁶⁵⁾. Due to associations with angioedema when combined with an ACE-I, neprilysin was subsequently combined with the ARB valsartan to produce the compound sacubitril-valsartan.

Results of the PARADIGM-HF trial demonstrated an approximate 20% reduction, not only in the composite primary endpoint, but in cardiovascular mortality, all-cause mortality and hospitalisation for HF ($p < 0.001$)⁽⁶⁴⁾. The trial was unique in its comparison of a novel compound compared with

standard therapy as opposed to placebo. Given the magnitude of the results, it is highly likely that sacubitril-valsartan will change the face of chronic HF therapy, with the potential to replace ACE-I as first line treatment. As a result of PARADIGM-HF, sacubitril-valsartan received European marketing authorisation in November 2015, with the first patients receiving treatment soon after. It's use for the treatment of chronic HF is expected to be included in the forthcoming update to the ESC guidance, with NICE approval/guidance expected in 2016.

1.1.7 Device Therapy

1.1.7.1 Implantable Cardioverter Defibrillator (ICD)

Although the majority of patients with symptomatic HF die as a result of pump failure, a significant proportion, particularly those with milder symptoms, suffer sudden cardiac death. Whilst the initiation of prognostically indicated medications may reduce this risk, they cannot prevent it.

The Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT)⁽⁶⁶⁾ enrolled 2521 patients with both non-ischaemic and ischaemic HF with an EF $\leq 35\%$, no prior symptomatic ventricular arrhythmia, and who were in NYHA functional class II or III. Patients were randomised to placebo, amiodarone, or an ICD, in addition to conventional treatment including an ACE-I or ARB, beta-blocker and MRA. ICD treatment led to a RRR in death of 23% over a median follow-up of 45.5 months. This benefit was additional to that gained with conventional treatment, although only 69% received beta blocker therapy and less than 20% were prescribed a MRA. These results, combined with additional positive results from several other trials^(67, 68) have led to the use of ICDs becoming an established treatment modality in HF management for those with persistent LVSD despite optimum medical therapy.

1.1.7.2 Cardiac Resynchronisation Therapy (CRT)

Several large randomised controlled trials have demonstrated significant mortality and morbidity benefits of CRT in HF patients who, despite optimum medical therapy, have persistent symptoms

(NYHA class II-IV), reduced ejection fraction ($EF \leq 35\%$), and are in sinus rhythm with evidence of dyssynchrony on ECG⁽⁶⁹⁻⁷²⁾. Two key placebo controlled randomised controlled trials, Comparison of Medical Therapy, Pacing and Defibrillation in Heart Failure (COMPANION) and Cardiac Resynchronisation in Heart Failure (CARE-HF), demonstrated RRR in death and HF hospitalisations of 24-52% in patients with NYHA class III-IV symptoms and QRS duration of $\geq 120\text{ms}$ ^(69, 70). Similar benefits were demonstrated in those with NYHA Class (II-III) symptoms in the Resynchronisation/Defibrillation for Ambulatory Heart Failure Trial (RAFT)⁽⁷²⁾. The Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronisation Therapy (MADIT-CRT) was unable to demonstrate mortality benefit in those with NYHA class I-II symptoms, but did show a RRR of 34% for the composite endpoint of death or HF hospitalisation⁽⁷¹⁾.

Despite such findings, CRT is not a benign therapy and can be associated with significant complications and the identification of patients most likely to benefit has been the focus of much work. Particular interest has been paid to the ECG parameters of QRS morphology and duration. Whilst there is clear evidence of benefit in those with LBBB and QRS duration of $\geq 150\text{ms}$, the evidence for those with non-LBBB or QRS duration of $< 150\text{ms}$ is less robust⁽⁶⁹⁻⁷³⁾.

In 2013, the results of the Cardiac Resynchronisation Therapy in Heart Failure with a Narrow QRS Complex (EchoCRT) trial were published. This study aimed to evaluate the benefits of CRT in patients with a QRS duration of $< 130\text{ms}$ and echocardiographic evidence of left ventricular dyssynchrony⁽³⁷⁾. The trial was terminated early due to the demonstration of increased mortality in the CRT treatment group compared with the control group (11.1% vs. 6.4% respectively, $p=0.02$). Much of this was powered by CV mortality which was 9.2% in the CRT treatment group compared with 4.2% in the control group ($p=0.004$). As a result of EchoCRT, guidance for the use of CRT changed such that the use of CRT pacing in patients with a QRS duration of $< 130\text{ms}$ is contraindicated.

Evidence for the use of CRT in those with atrial fibrillation is also less robust than in those with sinus rhythm^(74, 75). Studies have demonstrated, however, that provided strategies for biventricular capture are in place, patients in atrial fibrillation still derive benefit from CRT^(76, 77).

1.1.8 Mechanical Circulatory Support (MCS) & Cardiac Transplantation

Patients with HF who cannot be stabilised with medical or device therapy may benefit from mechanical circulatory support, either until recovery can be achieved or as a bridge to transplantation.

1.1.8.1 Intra-aortic Balloon Pump (IABP)

The use of intra-aortic balloon pumps in cardiogenic shock are not recommended and should be reserved for those patients who require circulatory support before surgical correction of specific acute mechanical problems (e.g. interventricular septal rupture and acute mitral regurgitation) or during severe acute myocarditis⁽²²⁾. There is no good evidence that an IABP is of benefit in other causes of cardiogenic shock, with recent trials examining their use in patients with acute myocardial ischaemia or infarction demonstrating no improvement in outcome⁽⁷⁸⁻⁸⁰⁾.

1.1.8.2 Percutaneous/Short Term Ventricular Assist Devices (pVAD)

Percutaneous ventricular assist devices (pVADs) are being increasingly used in patients with cardiogenic shock. They offer a means of instituting rapid and adequate cardiac support in patients with cardiogenic shock unresponsive to inotropes/vasopressors and IABP. Currently available pVADs include the TandemHeart, Impella and extracorporeal membrane circulation (ECMO). pVADs compensate for the loss of myocardial pump function, normalizing cardiac output and thus allowing physiologic perfusion of vital organs as compared with IABP, which solely decreases afterload and increases coronary blood flow (table 2)⁽⁸¹⁾.

	IABP	TandemHeart pVAD	Impella 2.5 Recover system	ECMO
Pump mechanism	Pneumatic	Centrifugal	Axial flow	Centrifugal flow
Insertion	Retrograde 7-9F balloon catheter into descending aorta via femoral artery	21F inflow cannula into left atrium via femoral vein and transseptal puncture and 15/17F outflow cannula into femoral artery	12F catheter (13F sheath) placed retrograde across the aortic valve via femoral artery	18–31F inflow cannula into the right atrium via femoral vein and 15-22F outflow cannula into descending aorta via femoral artery
Difficulty of insertion	+	++++	+++	++
Degree of support	+	+++	++	++++
Cardiac power output ^a	(↑ CO by 0.5 l/min)	(↑ CO by 3.5–4 l/min)	(↑ CO by 2.5 l/min)	(↑ CO to >4.5 l/min)
Time for implantation	10 min	25–65 min	11–25 min	10–15 min
Limb ischemia	+	+++	++	+++
Hemolysis	0	++	++++	+++
Bleeding risks	+	+++	++	++++
Contraindications	Moderate–severe AI/aortic stenosis, coagulopathy, severe sepsis	Peripheral arterial disease (may be placed with antegrade sideport for limb perfusion), RV failure	LV thrombus, ventricular septal defect, severe aortic stenosis, RV failure, peripheral arterial disease	Contraindication to anticoagulation, irreversible brain injury, terminal disease

Table 2: Comparison of IABP and pVADs for cardiogenic shock⁽⁸¹⁾

(AI, aortic insufficiency; CO, cardiac output; ECMO, extracorporeal membrane oxygenation; LV, left ventricular; RV, right ventricular).

There is considerable debate on the appropriate use of these devices given the difficulty of conducting randomised trials in patients with cardiogenic shock, lack of clear guidelines on indications, device selection, and cost-effective care of patients implanted with these devices. To date, most clinical trials have demonstrated that, whilst these devices do indeed provide superior haemodynamic support compared with IABP, this does not translate into improved early survival (table 3)^(82–88).

TRIAL	TREATMENT	CONDITION	PRIMARY	RESULT	SECONDARY
Tandem heart	IABP vs Tandem heart	Cardiogenic shock	Hemodynamic	Improved cardiac power index PCW, lactate	30 day mortality NS 45% IABP 43% VAD
Shock II trial	IABP vs Medical Rx	Cardiogenic shock	30 day survival	39.7% IABP 41.3% medical $P = 0.69$	1 yr survival 48% IABP 49% medical
ISAR shock	Impella 2.5 vs IABP	Cardiogenic shock	Hemodynamic 2 yr follow-up organ function	Improved CO, BP, PCW, lactate NS—46% mortality both groups 30 days	NS difference in LVEF, organ dysfunction, neurologic status 38% mortality both groups
Protect II trial	Impella 2.5 vs IABP	High risk PCI	30 day major adverse events	35.1% Impella 40.1% IABP $P = 0.227$	90 day MAE 40.6% Impella 49.3% IABP $P = 0.066$

Table 3: Clinical trials of pVADs vs. IABP support⁽⁸⁶⁾

At this time, the value of pVADs appears to lie in the benefit of allowing more time to help pursue revascularisation strategies or to allow for a complete evaluation of end-organ function and neurological status as a bridge to decisions regarding longer-term support⁽⁸¹⁾.

1.1.8.3 Implantable/Long Term Ventricular Assist Devices (VAD)

Heart transplantation has always been a limited therapeutic option for patients with end-stage chronic HF. The increasing number of patients with refractory, chronic HF and the declining numbers registering for organ donation have resulted in expanded waiting lists and prolonged waiting times for patients listed for heart transplantation. On 31st March 2015, there were 267 patients on the UK active transplant list, with only 181 transplants performed over the 2014/2015 period. With an overall median wait time of 134-256 days, the majority of patients are transplanted in high-urgency status, leaving little chance for patients listed for less urgent transplantation⁽⁸⁹⁾.

More recent data suggest that patients with ongoing LVAD support may have an improved survival on the transplant waiting list⁽⁹⁰⁾. Accordingly, MCS devices, particularly continuous-flow LVADs, are increasingly being viewed as a potential alternative to heart transplantation. Initially LVADs were developed for use as a short-term bridge to transplant approach, but they are now being used for months to years in patients who will either face a long-term wait on the transplant list or, in the USA and Europe, in patients who are not eligible for transplantation as permanent therapy or destination therapy^(91, 92).

In the UK, however, the NHS only supports the use of LVADs as a bridge to transplant. It does not fund 'destination therapy'. However, whilst the number of transplants remains lower than the number of patients currently on the transplant list, the number of patients on long term LVAD support has increased⁽⁹³⁾. In 2014/15 553 patients received a first long term VAD with duration of support ranging between 0 and 2390 days (median of approximately 520 days - an increase of 124 days (31%) compared with the same data from 2013/14)⁽⁹⁴⁾.

Whilst, on the surface, the use of LVADs appear an attractive solution to the diminishing availability of donor hearts, they are not without their complications and in 2014/15 the UK national unadjusted

rate of survival on a VAD 3 years after first continuous long-term device was 49%, compared with a 76% 5-year survival for cardiac transplantation^(89, 93). This, combined with questions regarding their cost effectiveness⁽⁹⁵⁾ means that it is unlikely that we will see the use of these devices for destination therapy in the UK anytime soon.

1.1.8.4 Cardiac Transplantation

Despite advances in medical and device therapies for chronic HF, prognosis remains poor for those who progress to end stage disease despite these interventions. Heart transplantation is an accepted treatment for selected patients with end-stage HF. Apart from the shortage of donor hearts, the main challenges in transplantation are ensuring appropriate patient selection and the subsequent consequences and complications of long term immunosuppressive therapy (i.e. antibody-mediated rejection, infection, hypertension, renal failure, malignancy and coronary artery vasculopathy).

Although controlled trials have never been conducted, there is a consensus that transplantation, provided that proper selection criteria are applied, significantly increases survival, exercise capacity, quality of life and return to work compared with conventional treatment and, as such, remains the definitive treatment for end-stage HF in appropriate patients^(96, 97).

1.1.9 Outcomes

Despite major advances in treatment, HF still has a prognosis worse than that of the majority of breast and prostate cancers and is associated with poor quality of life in many patients^(98, 99). Survival rates for patients who receive sub-optimal care are poor, with 40% of newly diagnosed patients dying within a year⁽¹⁰⁰⁾, and total annual mortality ranging from 10-40%, depending on disease severity.

In order to improve clinical outcomes (better quality of life, reduced mortality and morbidity and reduced hospital admission/readmission), strategies are required to improve diagnosis, treatment

and on-going support. Indeed, the National Heart Failure Audit⁽¹⁰¹⁻¹⁰³⁾ has consistently demonstrated that those patients identified early and treated on dedicated cardiology wards are more likely to receive evidence-based medication than those treated on general medical wards. In keeping with the wealth of evidence for medical therapy, such interventions are associated with a reduction in both in-hospital and post discharge mortality.

1.1.10 Monitoring

Despite significant advances in the treatment of chronic HF with both pharmacological and device therapy, hospitalisation rates remain unacceptably high, with 30-day readmission rates currently estimated at 20-25%⁽¹⁰⁴⁻¹⁰⁶⁾. Efforts to reduce this figure have focused on the use of improved outpatient monitoring techniques, ranging from sophisticated implantable devices to simple telephone support.

1.1.10.1 Remote Monitoring –with implanted devices

With the increased use of device therapy, so came the opportunity to utilize the sophisticated programming facilities within them for remote monitoring purposes. Moreover, with the development of implantable haemodynamic monitors, several studies have assessed whether early therapeutic intervention in response to physiological and/or hemodynamic changes would result in a reduction in HF hospitalisations. Several parameters associated with HF decompensation have been examined, including thoracic impedance, heart rate variability, right ventricular pressure, pulmonary artery pressure, left atrial pressure, as well as a combination of parameters. Whilst several meta-analyses examining the clinical benefit of these approaches have been encouraging⁽¹⁰⁷⁻¹⁰⁹⁾, prospective clinical trials have produced variable results⁽¹¹⁰⁻¹¹⁶⁾.

There has, however, been some success using the CardioMEMSTM wireless pulmonary artery monitoring system. Results of the CardioMEMS Heart Sensor Allows Monitoring of Pressure to

Improve Outcomes in NYHA Class III Patients (CHAMPION) trial demonstrated a 37% reduction in HF related hospitalisations over the six month study period in patients managed with daily pulmonary artery pressure monitoring in addition to standard care compared with standard care alone⁽¹¹⁷⁾. Subsequent data confirmed these results with a 33% reduction in HF related hospitalisations over an extended 18-month follow-up period⁽¹¹⁸⁾. In 2014, the CardioMEMS™ sensor became the first FDA approved implantable wireless heart monitoring device for the remote management of chronic HF patients.

More recently, the INfluence of home monitoring on mortality and morbidity in HF patients with IMpaired lEft ventricular function (IN-TIME) trial assessed the benefits of automatic multi-parameter telemonitoring for patients with HF treated with an ICD or CRT-D⁽¹¹⁹⁾. The primary outcome measure was a composite clinical score combining all-cause death, overnight hospital admission for HF, change in NYHA class, and change in patient global self-assessment. Results demonstrated that the odds of a worsening composite score were 37% higher in the control group compared to those managed with telemonitoring.

Despite these promising results, further research is required before considering widespread implementation of remote monitoring. The subset of the HF population that derives the most benefit from intensive monitoring, the best technology, and the optimum duration of monitoring, all need to be identified.

1.1.10.2 Remote Monitoring – without implanted devices

The monitoring of several non-invasive variables pertaining to HF prognosis and hospitalisation (HF symptoms, weight, ECG and blood pressure) has been compared with usual care in several trials of remote monitoring^(120, 121). To date no variable, or combination of variables has been shown to significantly improve outcomes.

1.1.10.3 Telephone Support

Several randomised controlled trials have studied the benefits of telephone support in preventing HF hospitalisations⁽¹²²⁻¹²⁶⁾. Although recent meta-analyses of RCTs suggests that structured telephone support in addition to conventional care may reduce the risk of hospitalization^(127, 128), variation in interventions and usual care has meant that definitive conclusions regarding both the healthcare and economic benefits of such interventions are currently lacking.

1.1.10.4 Biomarkers

With the identification of prognostic biomarkers for HF, the potential for these markers to detect decompensation prior to the development of clinical signs and symptoms has generated much interest⁽¹²⁹⁻¹³⁸⁾ and is the focus of this thesis.

1.2. Biomarkers

1.2.1 Definition

Biomarkers are 'cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids.'⁽¹³⁹⁾ More recently, the National Institute of Health (NIH) definition of a biomarker has expanded this definition to include any biological characteristic that can be objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or the response to therapeutic intervention⁽¹⁴⁰⁾. For the cardiovascular system, there is a wide range of techniques used to gain information about the heart in both the healthy and disease state. These may involve measurements directly on biological media or techniques, such as echocardiography, that measure changes in the structure or function of the heart.

Although many biological markers fulfil this definition and are utilised extensively in research settings, only those proven to cost-effectively alter medical management and outcomes are subsequently integrated into everyday clinical practice.

The characteristics of an ideal biomarker include:

- It should be visible early, prior to histopathological changes, and should be indicative after active damage.
- It should be sensitive, but it should also correlate with the severity of damage.
- It should be accessible in the peripheral tissue.
- It should be analytically stable in tissue so it can be measured after some time has passed.
- It should be associated with a known mechanism.
- It should be able to localize damage to specific tissues.

1.2.2 Clinical value of biomarkers

There are two major types of biomarker: those of exposure, which are used in predicting risk of

developing a disease, and those of disease⁽¹⁴¹⁾, which may be used for:

- Diagnosis of clinical disease in patients with uncertain symptoms
- Screening for pre-clinical disease in asymptomatic patients
- Risk stratification in patients with clinical disease
- Therapeutic guidance in selection or titration of agents in patients with known disease

Used appropriately, biomarker measurement can influence health outcomes in several ways (figure 10).

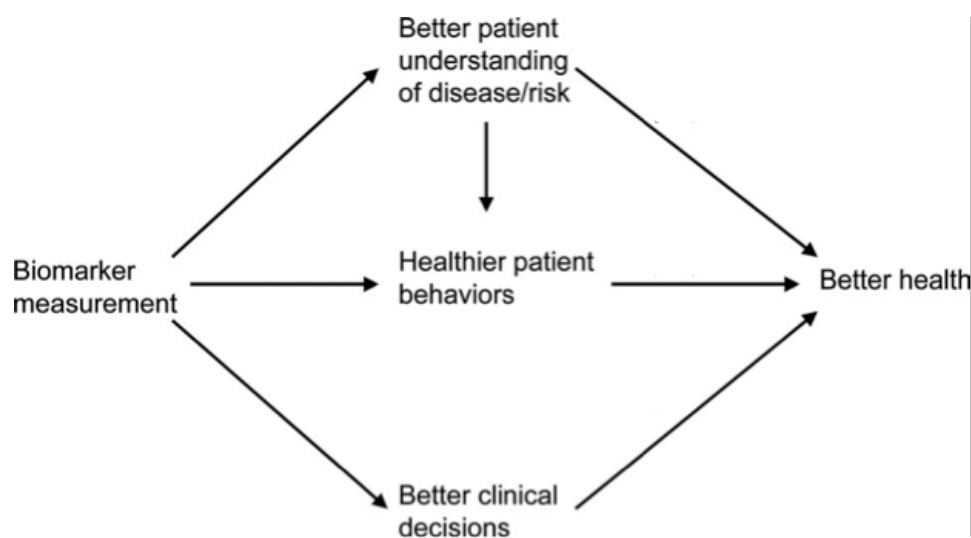


Figure 10: Influence of biomarker measurement on health outcomes⁽¹⁴²⁾

1.2.2.1 Diagnosis

In the diagnostic effort, collection of information from various sources helps to achieve the ultimate goal of increasing the probability of a given diagnosis. If a biomarker is to be used to aid the diagnostic conundrum, it should be sensitive and specific and have a high predictive value. A highly sensitive test will be positive in nearly all patients with the disease, but it may also be positive in many patients without the disease. Therefore, to be of clinical value, a test with high sensitivity should also have high specificity; in other words, most patients without the disease should have negative test results. For predicting the likelihood of disease on the basis of the test result, the appropriate measures are positive and negative predictive values.

Most biological markers, however, are not simply present or absent, but have wide ranges of concentrations that overlap those with disease and those without it. The likelihood of disease presence typically increases progressively with increasing levels, so various cut-off points must be evaluated for their ability to detect disease. Cut-off points with high sensitivity, producing few false negative results, are used when the consequences of missing a potential case are severe, whereas highly specific cut-off points, producing few false positive results are used to avoid mislabelling a person who is, in fact, free of the disease.

The diagnostic performance of a biomarker is additionally influenced by disease prevalence and an assay with identical sensitivity and specificity may have different utility in the diagnosis of a common disease compared with a rare disease. It is therefore necessary to consider the likelihood of the disease being present when using a biomarker to diagnose that disease. In statistical terms, this requires knowledge of the pre-test probability, the likelihood ratio and the post-test probability:

1.2.2.1.1 Pre-test probability

The pre-test probability describes the proportion of people, in the population at risk, who have the disease at a specific time or time interval. In other words, it is the probability, before the diagnostic test is performed, that a patient has the disease.

The probability of the target disorder, usually abbreviated $P(D+)$, can be calculated as the proportion of patients with the target disorder, out of all the patients with the symptoms(s), both those with and without the disorder:

$$P(D+) = D+ / (D+ + D-)$$

where $D+$ indicates the number of patients with target disorder, $D-$ indicates the number of patients without target disorder, and $P(D+)$ is the probability of the target disorder.

1.2.2.1.2 Likelihood ratios

Likelihood ratios (LR) are used to assess two things: the potential utility of a diagnostic test, and how likely it is that a patient has a disease or condition. LRs are basically a ratio of the probability that a test result is correct to the probability that the test result is incorrect and provide a direct estimate of how much a test result will change the odds of having a disease.

Likelihood ratios are calculated from sensitivity and specificity of the test, and therefore do not depend on prevalence in the reference group. In contrast to positive or negative predictive values, they do not change with changed pre-test probability.

Because tests can be positive or negative, there are at least two likelihood ratios for each test. The likelihood ratio of a positive test result (LR+) is sensitivity divided by (1- specificity).

$$(LR+) = \frac{\text{True positives}}{\text{False positives}} = \frac{\text{Sensitivity}}{(1-\text{Specificity})}$$

The likelihood ratio of a negative test result (LR-) is (1- sensitivity) divided by specificity.

$$(LR-) = \frac{\text{False negatives}}{\text{True negatives}} = \frac{(1-\text{Sensitivity})}{\text{Specificity}}$$

The further away a likelihood ratio (LR) is from 1, the stronger the evidence for the presence or absence of disease. LR >1 indicates that the test result increases the probability of disease. LR <1 indicates that the test result decreases the probability of disease.

1.2.2.1.3 Post-test probability

The post-test probability is the probability, after the diagnostic test is performed, that a patient has the disease. It describes the proportion of patients testing positive who truly have the disease and is

similar to the positive predictive value, but also includes a patient based probability of having disease. The post-test probability can be calculated using a simple Fagan Nomogram (figure 11).

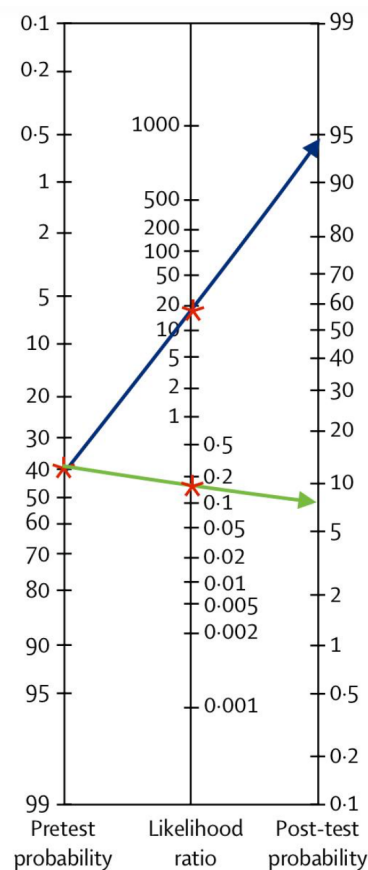


Figure 11: Fagan nomogram showing post-test probabilities for a positive (blue) and negative (green) diagnostic test result.

1.2.2.2 Screening

The principles of a screening programme are to detect disease in a population at an early stage, enabling earlier intervention and management in the hope of reducing mortality and morbidity from the disease. Although screening may lead to an earlier diagnosis, not all screening tests have been shown to benefit; over diagnosis, misdiagnosis, and creating a false sense of security are some potential adverse effects of screening. For these reasons, a test used in a screening programme must have good sensitivity in addition to acceptable specificity.

Guidelines on screening were first published by the World Health Organisation in 1968⁽¹⁴³⁾, but the principles are still applicable today:

- The condition should be an important health problem.

- There should be a treatment for the condition.
- Facilities for diagnosis and treatment should be available.
- There should be a latent stage of the disease.
- There should be a test or examination for the condition.
- The test should be acceptable to the population.
- The natural history of the disease should be adequately understood.
- There should be an agreed policy on whom to treat.
- The total cost of finding a case should be economically balanced in relation to medical expenditure.
- Case finding should be a continuous process, not just a "once and for all" project.

It is for these reasons that few biomarkers are currently used for mass population screening but are used only in selected cases where the pre-test probability of a disease being present is already high.

1.2.2.3 Prognosis

Many patients newly diagnosed with disease pose questions regarding prognosis. Predicting the course of a particular disease in a particular individual remains challenging due to the contribution of various factors including patient demographic, co-morbidities and disease specific features. The use of biomarkers to prognosticate disease states provides a somewhat objective measurement upon which to base treatment strategies and patient education/counselling.

For a biomarker to be used for prognostic purposes, it should parallel changes either in disease state or those occurring as a result of therapeutic intervention. Moreover, the prognostic significance of a biomarker may vary depending on the specific end-point tested and it is for this reason that multi-marker schemes may be required to encompass different outcomes of interest. Studies examining the prognostic ability of a biomarker should begin at a defined point of time in the disease course,

follow up patients for an adequate amount of time, and measure all relevant outcomes. In addition:

- The study population should include all those with a disease in a defined population.
- Patients should be followed up from the same defined point in the disease course to ensure a precise estimate of prognosis.
- Patients must be followed up for long enough that most important outcomes have occurred.
- Prognostic estimates should include all aspects of a disease that are important to patients, including quality of life measures and not just death or recovery.

1.2.2.4 Monitoring

With the ability to prognosticate, comes the potential to monitor disease progression. The use of serial sampling to detect important changes prior to the onset of symptoms is an attractive method for selecting patients for early intervention and prevention of costly hospital admissions. From a clinical perspective, this method is easily applied to biomarkers measurable in body tissue such as blood and urine, which are both readily available and accessible via acceptable sampling methods.

Inherent to any biomarkers ability to accurately monitor disease progression is the extent to which measurements vary under 'normal' conditions.

1.2.3 Biological variability of biomarkers

Although biomarkers have numerous advantages, variability is a major concern. Several factors within the environment, the individual and within the test itself contribute to variability and various measures can be taken in order to reduce the impact of these on the final result (table 4)⁽¹⁴⁴⁾. While measurement error is always a concern with biomarkers, other important factors may explain individual or group variability. Consideration of the sources of variability in the measurement of a biomarker is essential to decrease the potential for misclassification of diagnosis and/or prognosis.

Inter-individual variability can result from environmental exposures, drugs, or other effect modifiers

that can increase or decrease the result of the biomarker under consideration. Variability can also be attributed to the effects of factors such as concurrent disease states or other personal characteristics. The amount of body fat, for example, can influence the biological measurement of several biomarkers.

Intra-individual variability, on the other hand, is usually related to laboratory errors or other conditions, or exposures unique to the individual.

Preanalytical Variability	Analytical Variability	Steps to Reduce Variability
Sample and assay related	Interlaboratory variability	For clinical chemistry laboratory
Type of specimen	Analytical platforms	Use of a reference lab and reference standard
Type of sample	Lot-lot variability	Replicate measurements
24-h vs single morning void	Reagents	Add phantoms (dummy ids) to samples
Sample processing	Calibration functions	Limit multiple lots
Anticoagulant		Freeze samples immediately
Stabilizing agent	Intralaboratory variability	Avoid repeated freeze and thaw cycles
Temperature	Personnel-related	Regular calibration of instruments
Endogenous degrading enzymes	Interreader and intrareader	Assess interassay and intra-assay precision at low and high levels
Freeze-thaw cycles	Temporal drifts	Optimal is $<1/4$ (CV_i ; within-subject variability)
Sample storage	Lot-lot variability	Desirable is $<1/2$ CV_i
Assay related		Minimal acceptable is $<3/4$ CV_i
Minimal detection limit		Assess bias (based on CV_i and CV_b , where CV_b is between-subject variability)
		Optimal is <0.125 ($CV_i + CV_b$) ^{1/2}
Image acquisition		Desirable is <0.25 ($CV_i + CV_b$) ^{1/2}
Interobserver		Minimal acceptable is <0.375 ($CV_i + CV_b$) ^{1/2}
Intraobserver		
		Regular laboratory supervision and assessment of drifts
Biological (subject related)		Develop reference ranges
Intraindividual		Assess impact of covariates on analyte values
Diurnal		
Day-to-day		
Seasonal		
Menstrual		
Fasting state		
Beat-to-beat (for imaging studies)		
Interindividual		
Age		
Gender		
Race		
Pregnancy		
Menopausal status		
Drugs		
Diet		
Exercise		

Table 4: Factors affecting and steps to reduce variability⁽¹⁴⁴⁾

In the study of biological variation, several different indices can be calculated in order to estimate these variances⁽¹⁴⁵⁾:

- Total Coefficient of Variation (CV_t) - composed of both analytic and biologic variation.
- Analytical Coefficient of Variation (CV_a) - the variation that exists within the measurement process. This is normally kept to a small fraction of the intra and inter-individual variances.
- Intra-individual Coefficient of Variation (CV_i) - the random variation that occurs around a homeostatic set point within an individual. This can be calculated according to the formula:
$$CV_i = (CV_t^2 - CV_a^2)^{1/2}.$$
- Inter-individual Coefficient of Variation (CV_g) – the variation between individuals, calculated as a ratio of standard deviation over mean.
- Index of Individuality – the ratio of intra-individual to inter-individual variation (CV_i/CV_g). High index of individuality (>1.4) occurs when there is little variance between individuals relative to the population as a whole. Low index of individuality (<0.6) occurs when there is significant difference between individuals relative to the population and indicates that single concentrations cannot be effectively compared to a population-based reference range⁽¹⁴⁶⁾.

Inherent to the use of any biomarker derived from body tissue in a clinical setting is the understanding of how these variables affect concentrations both in the healthy and disease state. A single measurement of an analyte represents a concentration that independently randomly fluctuates around a normal homeostatic set point. However, knowledge of the temporal changes following acute episodes of disease is a necessary prerequisite for the selection of the most appropriate test(s) to aid diagnosis and is vital for the correct interpretation of results. As with all clinical analytes, the biomarkers of disease change with time and stages of disease. Consequently, consideration of the rate at which change occurs in disease state is important for clinical management and assessment of prognosis/monitoring.

One way in which to estimate the importance of such changes is to calculate the degree to which a change in biomarker measurement constitutes statistical significance – the Reference Change Value (RCV). This can be calculated using the formula

$$RCV = Z \times 2^{1/2} (CV_i^2 + CV_a^2)^{1/2}$$

where Z (the 95% confidence interval Z score) is 1.96. This level of RCV reflects the minimum percentage change in serial results that is, with 95% confidence, different from the combined analytical and biological variation. Thus, the lower the RCV, the more likely it is a biomarker will be useful for monitoring purposes.

1.2.4 Biomarkers of chronic heart failure

In the evolution of the heart from an at-risk but structurally normal organ to ventricular dysfunction and eventual progression into symptomatic HF, various remodelling and neurohormonal activation pathways activated may be leveraged for biological monitoring. Consequently, there are increasing numbers of measurable injury, remodelling and neurohormonal activation peptides, whose concentrations might relay important information about diagnosis, prognosis and pathophysiological processes (table 5).

Myocardial insult	Neurohormonal activation	Remodelling
<i>Myocyte stretch</i> NTproBNP BNP MRproANP Apelin	<i>Renin angiotensin system</i> Renin Angiotensin II Aldosterone	<i>Inflammation</i> CRP, TNFα, Fas Interleukins Osteoprotegerin Adiponectin
<i>Myocardial injury</i> Troponin T Troponin I	<i>Sympathetic nervous system</i> Noradrenaline Chromogranin A	<i>Hypertrophy/Fibrosis</i> Matrix metalloproteinases Collagen propeptides Galectin-3 sST2
<i>Oxidative stress</i> Myeloperoxidase Oxidised low-density Lipoproteins MRproADM	<i>Arginine vasopressin system</i> Arginine vasopressin	<i>Apoptosis</i> Growth differentiation factor 15

Table 5: Cardiac biomarkers of myocardial insult, neurohormonal activation and remodelling. Adapted from ⁽¹⁴⁷⁾

1.3. B-type Natriuretic Peptides

Of the recognised potential biomarkers, the B-type natriuretic peptides are the most extensively studied and currently utilised. Whilst the ability of the heart to induce natriuresis in response to atrial stretch was first recognised in the 1950's⁽¹⁴⁸⁾, it was not until the early 1980's that the first of these, atrial natriuretic peptide (ANP), was formally identified⁽¹⁴⁹⁾. In 1988 it was further discovered that brain natriuretic peptide (BNP), a peptide initially isolated from pig brain but primarily synthesised by the ventricular myocardium⁽¹⁵⁰⁾, produced similar natriuretic and diuretic responses as ANP. Using models of experimental HF and from human subjects, it was demonstrated that both ANP and BNP were produced and released in response to myocardial stretch and increased filling pressures observed in the pathophysiology of HF⁽¹⁵¹⁻¹⁵⁴⁾.

1.3.1 BNP and NTproBNP release and cardiovascular effects

BNP production in normal healthy individuals is minimal. In response to myocyte stretch, however, the BNP gene, natriuretic peptide precursor B (NPPB), expressed within the heart, produces an initial 134 amino acid pre-proBNP precursor peptide. Removal of a 26-amino acid signal peptide results in the pro-hormone, proBNP. ProBNP is then cleaved into the biologically active BNP and the biologically inactive N-terminal peptide (NTproBNP) (figure 12).

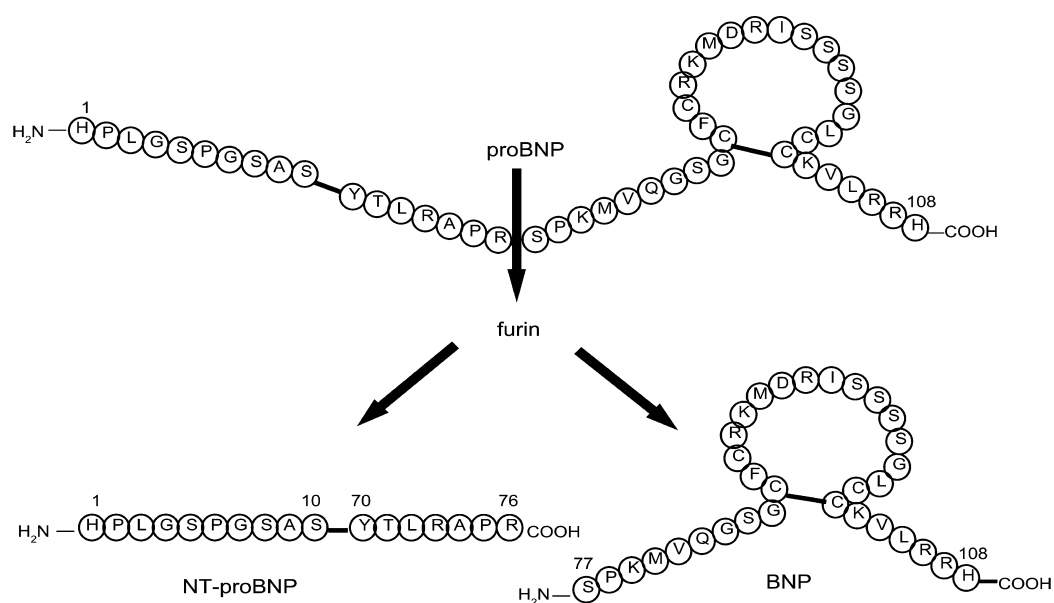


Figure 12: Enzymatic cleavage of proBNP into biologically active BNP and the inactive NTproBNP fragment⁽¹⁵⁵⁾

Subsequent binding of the active peptide to its receptor, natriuretic peptide receptor A (NPR-A) activates the production of the second messenger cyclic guanosine monophosphate (cGMP), which mediates the effects of BNP. The net result is that of inhibition of sodium reabsorption in the proximal convoluted tubule and natriuresis, increase in glomerular filtration and diuresis, relaxation of vascular smooth muscle and vasodilatation. Overall this leads to reduced blood pressure, ventricular preload and suppression of the renin-angiotensin-aldosterone system (figure 13)⁽¹⁵⁶⁾. In addition, there is inhibition of fibroblast activation and ultimately reverse/prevention of remodelling of the ventricle.

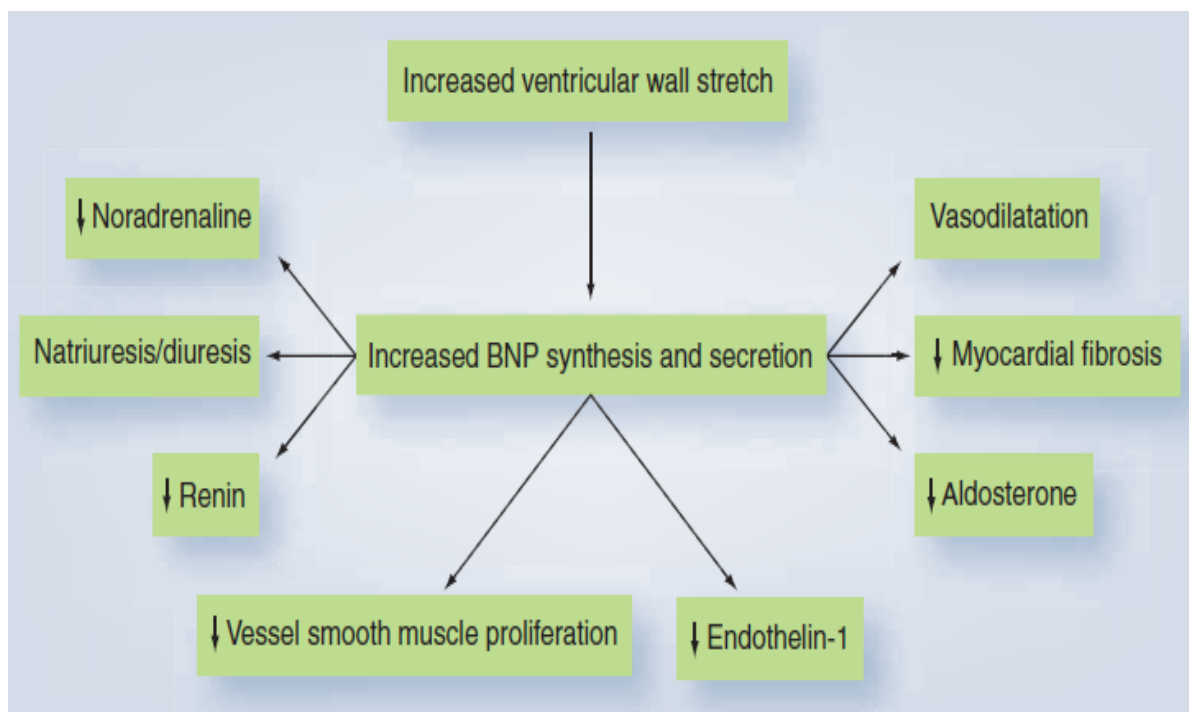


Figure 13: Actions of BNP⁽¹⁵⁶⁾

Regulation of BNP production occurs at the level of gene expression, and can increase rapidly in response to a trigger stimulus⁽¹⁵⁷⁾. Although this is primarily myocyte stretch, other stimuli such as tachycardia, left ventricular hypertrophy, ischaemia, hypoxaemia, sepsis, thyroid dysfunction, renal dysfunction, pulmonary embolism and liver cirrhosis can also trigger gene expression and thereby BNP production⁽¹⁵⁸⁻¹⁶⁰⁾. Actual circulating levels are, however, influenced by many factors including age, gender and weight⁽¹⁶¹⁾, with obesity and prior treatment with prognostic HF medications being associated with lower levels⁽¹⁶²⁾.

Circulating BNP is degraded by the membrane metallo-endopeptidase (MME) enzyme, neprilysin. As discussed in section 1.1.6.9, the neprilysin inhibitor, sacubitril, forms part of the novel compound sacubitril-valsartan, which has demonstrated significant benefits over and above those of ACE-I for the treatment of chronic HF secondary to left ventricular systolic dysfunction⁽⁶⁴⁾. The introduction of such therapy will, consequently, alter the utility of BNP in the HF setting, with higher concentrations of BNP present in those treated with sacubitril-valsartan. The concentrations and therefore use of NTproBNP will, however, remain unaffected.

1.3.2 BNP and NTproBNP for diagnosis of heart failure

During the early part of the last decade, studies into the use of BNP and NTproBNP as a diagnostic tools began advocating their use in addition to signs and symptoms in patients suspected of having HF^(163, 164). Such studies indicated that raised concentrations were both more sensitive and specific at distinguishing HF from other causes of dyspnoea than either LVEF or ANP alone⁽¹⁶³⁻¹⁶⁷⁾. Moreover, it was observed that levels were highest in those with decompensated HF, intermediate in those with known left ventricular dysfunction but no acute exacerbation and lowest in those without HF.

It was the results of the Breathing Not Properly Study⁽¹⁶⁸⁾ in 2002 that first illustrated the importance of BNP in the diagnosis of HF and led to the widespread clinical use of BNP. In particular, with a negative predictive value (NPV) of 87% with BNP levels less than 100pg/ml and an NPV of 97% with NTproBNP levels less than 125pg/ml natriuretic peptide measurement is especially useful at ruling out HF⁽¹⁶³⁾. Since this time, numerous studies have corroborated the use of the natriuretic peptides for HF diagnosis^(163, 164, 169-171) with NICE first advocating its use as a rule-out test for HF in 2003⁽¹⁷²⁾.

As diagnostic tools, it is anticipated that both BNP and NTproBNP will remain valid at this time. Given it is unlikely that neprilysin inhibition will have commenced in those without a formal diagnosis of HF, the current levels of both BNP and NTproBNP used to rule out a diagnosis of HF can still be

considered reliable. Should future applications of neprilysin inhibition result in the potential for patients without HF to be treated, then the use of BNP will need to be revised and NTproBNP is likely to become the natriuretic peptide biomarker of choice.

1.3.3 BNP and NTproBNP for prognosis of heart failure

Given the relationship with left ventricular stretch and filling pressures, it is unsurprising that circulating BNP and NTproBNP levels are not only associated with diagnostic accuracy, but also with prognostic accuracy in patients with HF⁽¹⁷³⁻¹⁷⁷⁾. In these patients, higher concentrations of BNP and NTproBNP are associated with increased cardiovascular and all-cause mortality, independent of age, NYHA class or LVEF. In addition, raised BNP and NTproBNP levels are also associated with readmission for HF and outcomes after presentation to the emergency department⁽¹⁷⁸⁾. Patients with persistently high concentrations of BNP and NTproBNP despite aggressive treatment are at especially high risk for adverse outcomes⁽¹⁷⁶⁾.

The results of the PARADIGM-HF trial⁽⁶⁴⁾ will result in an increased use of the angiotensin-neprilysin inhibitor, sacubitril-valsartan. As previously described, inhibition of this enzyme prevents the natural degradation of BNP, resulting in artificially elevated concentrations. As a prognostic marker, BNP may have limited use in patients treated with sacubitril-valsartan and, as such, NTproBNP should be the natriuretic peptide of choice for prognostication in such patients.

1.3.4 BNP and NTproBNP for monitoring heart failure

Given their correlation with prognosis, it is logical to assume that serial measurement of BNP or NTproBNP might prove useful in optimising pharmacotherapy for chronic HF and in so doing, improve patient outcomes. Initial small studies proved inconclusive in this respect. Whilst Troughton et al⁽¹⁷⁹⁾ found that natriuretic peptide guided treatment resulted in a reduction of total CV events and a delay in time to first event compared with intensive clinically guided treatment, Beck-da-Silva

and colleagues were unable to demonstrate any improvement in beta-blocker dosing or a reduction in hospitalisations or mortality with such a strategy⁽¹⁸⁰⁾. In order to fully elucidate the potential of natriuretic peptides in this respect, several larger randomised clinical trials have evaluated whether adjustment of therapy guided by natriuretic peptide levels, compared with conventional strategies based on clinical assessment, resulted in a favourable reduction in mortality/morbidity^(129, 130, 132, 133, 135, 181-184) (table 6)⁽¹⁸⁵⁾.

In 2006, a New Zealand group of researchers proposed the NTproBNP-AssisTed Treatment To LEssen Serial CARdiac Readmissions and Death (BATTLESCARRED) trial to assess the benefit of natriuretic peptide guided therapy⁽¹⁸³⁾. Compared with usual care in the community, intensive management *with or without* serial natriuretic peptide monitoring improved mortality at one year in patients with chronic HF but at two and three years, there was no significant difference between treatments overall. Sub group analysis, however, did reveal that mortality and hospital admission rates were reduced with peptide-guided therapy compared with usual care in patients younger than seventy-five years. No benefits were observed in patients older than seventy-five years.

The Trial of Intensified Versus Standard Medical Therapy in Elderly Patients with Congestive Heart Failure (TIME-CHF) was subsequently designed to evaluate the use of NTproBNP guided therapy in younger patients (60 to 74 years) compared with an older cohort (75 years or greater)⁽¹⁸²⁾. Mortality was significantly reduced in the younger age group but no such benefit was observed in the older age group. Although those in the NTproBNP guided therapy treatment arm received significantly higher doses of ACE-I or ARB and beta-blockers, NTproBNP levels decreased in all treatment groups with no significant difference in NTproBNP reduction between the groups.

It was proposed, therefore, that the benefits observed might reflect better pharmacotherapy than that of absolute reduction in natriuretic peptide concentration. Further studies have demonstrated

Trial	Treatment		Control (n)	Type of Peptide	Ischaemic					Loop					Detsky Quality Score	
	Year	(n)			Women (%)	Age (yrs)	Aetiology (%)	HTN (%)	DM (%)	NYHA class	LVEF (%)	ACE-I or ARB (%)	MRA (%)	Diuretic (%)		Follow-up (yrs)
Troughton¹³	2000	33	36	NT-proBNP	23.2	70.1	73.9	65.2	13.0	2.0	27.0	NA	NA	NA	0.79	90%
Beck da Silva¹⁴	2005	21	20	BNP	65.9	65.0	41.5	NA	NA	2.5	22.4	NA	NA	NA	0.33	90%
STARS-BNP¹⁵	2007	110	110	BNP	42.3	65.5	46.8	NA	NA	2.3	30.9	99.1	98.2	23.2	1.25	85%
TIME-CHF¹⁶	2009	251	248	NT-proBNP	34.5	76.5	57.5	70.9	34.5	0.0	29.8	94.8	78.6	40.5	1.5	81%
BATTLESCARRED¹⁷	2010	121	243	NT-proBNP	36.0	75.7	59.1	43.7	17.9	2.1	38.7	NA	NA	NA	3	90%
SIGNAL-HF¹⁸	2010	126	124	NT-proBNP	28.8	77.5	NA	54.8	20.0	2.4	32.0	93.6	77.6	20.0	0.75	86%
PRIMA¹⁹	2010	174	171	NT-proBNP	42.9	72.2	21.2	NA	NA	2.1	35.8	56.5	55.9	18.6	2	86%
Anguita²⁰	2010	30	30	BNP	NA	NA	NA	NA	NA	0.0	NA	NA	NA	NA	1.33	NA
Berger²¹	2010	92	186	NT-proBNP	35.3	71.3	69.4	72.3	45.0	0.0	NA	NA	NA	NA	1	85%
STARBRITE²²	2011	65	65	BNP	30.0	61.0	40.8	NA	NA	0.0	20.0	90.8	NA	67.7	0.5	81%
UPSTEP²³	2011	147	132	BNP	27.2	70.9	NA	28.0	31.2	2.8	NA	100.0	93.9	57.0	1	90%
PROTECT²⁴	2011	75	76	NT-proBNP	15.2	63.3	56.3	52.3	41.1	0.0	26.9	81.5	96.0	41.7	0.83	90%

Table 6: Summary of trials examining the use of the B-Type Natriuretic Peptides for monitoring chronic heart failure

similar effects on pharmacotherapy but results pertaining to improvement in mortality/morbidity have been less convincing^(133, 135, 181, 184). With an almost equal number of studies showing 'positive' and 'neutral' results, and in an overall relatively small number of patients, three meta-analyses have attempted to consolidate the results⁽¹⁸⁵⁻¹⁸⁷⁾. Pooled results indicate that natriuretic peptide guided therapy results in a reduction in all-cause mortality, but not all-cause hospitalisation (figures 14 and 15). The exact effect of better pharmacotherapy alone on these results remains undetermined.

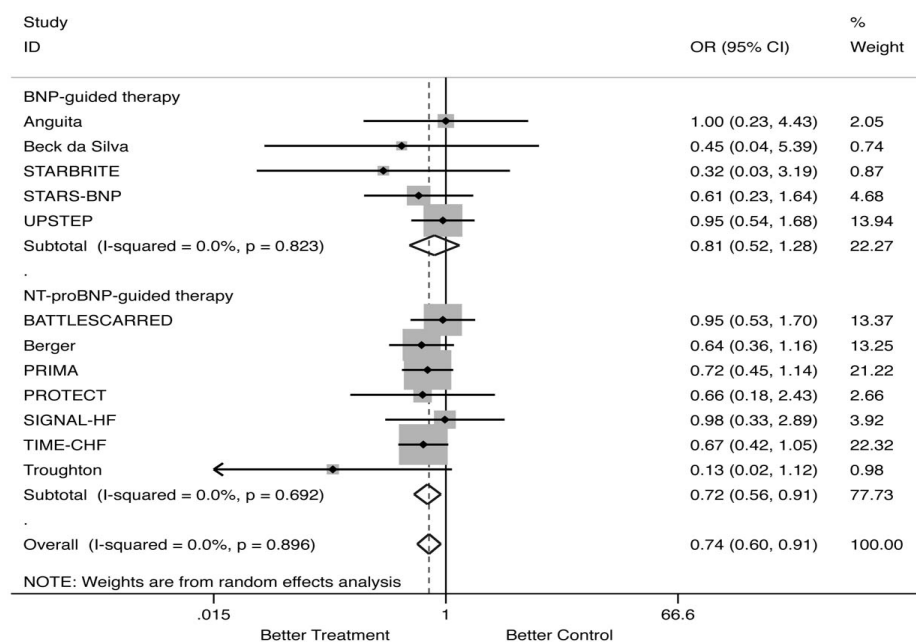


Figure 14: Odds ratio for all-cause mortality in monitoring trials⁽¹⁸⁵⁾

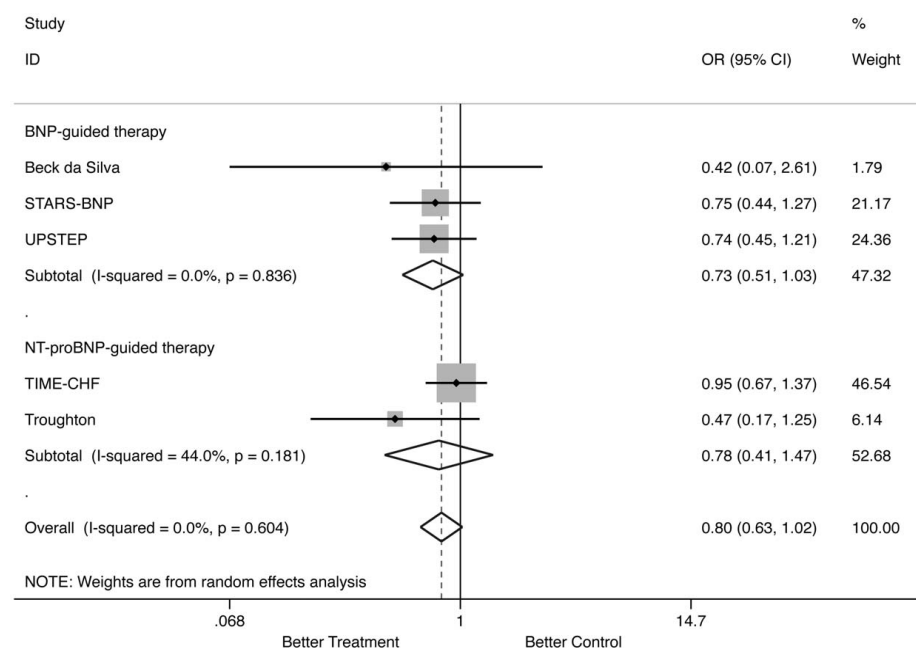


Figure 15: Odds ratio of all-cause hospitalisation in monitoring trials⁽¹⁸⁵⁾

Given the recent findings of the PARADIGM-HF trial, it is anticipated that sacubitril-valsartan will become an established therapy for the treatment of chronic HF. Clearly no trials have assessed the use of B type natriuretic peptide monitoring in patients treated with angiotensin-neprilysin inhibitors. As with prognostication, the resulting artificially high BNP concentrations will prevent sensible interpretation of results. Combined with the data presented above, the future of BNP as a monitoring tool is, therefore, in serious doubt.

1.3.5 Biological variability of BNP and NTproBNP:

Several small studies now indicate that the biological variability of the B-type natriuretic peptides is probably too high for them to be used accurately for the purpose of monitoring to detect clinically significant changes in disease progression⁽¹⁸⁸⁻¹⁹²⁾. In these studies, the mean individual co-efficient of variation (CV_i) for weekly sampling in chronic HF patients ranges from 21% to 33% for NTproBNP and 22% to 50% for BNP. This results in reference change values for these peptides of 49% to 92% for NTproBNP and 66% to 138% for BNP.

Given that it is the prognostic value of a biomarker that predicts its potential as a monitoring tool for chronic HF, there are several novel biomarkers to which assays are now available, that may prove more useful in this respect. It is the aim of this MD to examine 4 novel biomarkers, Mid-Regional Pro-adrenomedullin (MRproADM), Apelin, sST2 and Galectin-3, in respect to their potential for monitoring.

1.4. Mid-Regional pro-Adrenomedullin (MRproADM)

Adrenomedullin (ADM) is a 52-amino acid peptide with potent endogenous vasodilatory and natriuretic properties⁽¹⁹³⁾. Originally isolated in 1993 from extracts of human pheochromocytoma cells in the adrenal medulla⁽¹⁹⁴⁾, it has subsequently been isolated in a wide variety of tissues including brain, lung, kidney, gastrointestinal tract and the heart⁽¹⁹⁵⁾. It shows 27% homology with calcitonin gene related peptide (CGRP)⁽¹⁹⁵⁾ and as such belongs to the calcitonin superfamily of peptides.

Reliable quantification of the peptide itself is hampered by both its short plasma half-life (approximately 22 minutes), its binding to plasma proteins and degradation via the membrane metallo-endopeptidase (MME) enzyme, neprilysin. As with the natriuretic peptides, ADM is derived from a larger precursor peptide, preproadrenomedullin (preproADM). By post-translational processing, two smaller biologically inactive proADM fragments are produced: one mid-regional part (MRproADM) and the COOH terminus. Work by Struck et al⁽¹⁹⁶⁾ indicated that, compared with ADM, the MRproADM fragment was not only stable in human plasma, but that the released amounts directly reflected those of ADM. Later, the same group developed an immunoluminometric assay to MRproADM, which does not suffer from the issues with plasma half-life or plasma protein binding. Concentrations are also not affected by the use of angiotensin-neprilysin inhibitors⁽¹⁹⁷⁾. Much of the extended work on the use of ADM as a biomarker for HF has concentrated on utilising MRproADM as the marker of choice and should not be invalidated or altered by the introduction of such therapy into routine HF care.

1.4.1 Adrenomedullin release and cardiovascular effects

The main sites of production of ADM are endothelium and vascular smooth muscle^(198, 199). Its production is upregulated by several factors such as oxidative stress, pro-inflammatory cytokines, angiotensin II (Ang II), hypoxia, hyperglycaemia, natriuretic peptide, and aldosterone⁽²⁰⁰⁾.

In vitro, ADM dilates blood vessels of different vascular beds in different animal species. Early animal models examining the action of ADM on the cardiovascular system suggested that ADM might act as a regulator of systemic vascular tone, with rats administered intravenous ADM displaying a marked dose-dependent decrease in mean arterial blood pressure⁽¹⁹⁴⁾. The mechanism of vasodilatory effect of ADM has been addressed in many studies and the results differ depending on the experimental model. In general, most data indicate that:

- ADM induces endothelium independent relaxation by acting on CGRP₁ receptors and elevating cyclic adenosine monophosphate (cAMP) levels in vascular smooth muscle cells.
- ADM secretion by endothelial cells may function as an endothelium-derived relaxing factor.
- ADM may also activate potassium channels in smooth muscle cells causing cell hyperpolarization⁽²⁰¹⁾.
- ADM binds to specific receptors in endothelial cells and elicits endothelium-dependent vasodilatation mediated by nitric oxide (NO)⁽²⁰²⁾, endothelium-derived hyperpolarizing factor⁽²⁰³⁾, and/or vasodilatory prostanoids⁽²⁰⁴⁾.
- ADM activates endothelial nitric oxide synthase (eNOS) by at least two mechanisms. First, ADM elevates intracellular calcium levels, which increases eNOS activity⁽²⁰⁵⁾. Second, ADM activates phosphatidylinositol 3-kinase (PI3K) and protein kinase B/Akt, which phosphorylates eNOS and increases its activity even at low calcium concentrations⁽²⁰⁶⁾.
- ADM may also inhibit vasoconstrictor endothelin-1 production by endothelial cells⁽²⁰⁷⁾.

Further studies have demonstrated the ability of intravenously infused ADM to decrease cardiac preload and afterload and improve contractility of the myocardium with a resultant increase in cardiac output^(208, 209). This is likely the net result of reduction in peripheral resistance, with a

consequent decrease in afterload. Although decreased peripheral resistance and blood pressure induces reflex tachycardia, heart rate increases to a lower extent than after administration of other vasodilators that induce comparable hypotension. Combined with both cAMP dependent and independent increases in myocardial inotropy^(210, 211) the net result is one of increased cardiac output. Further work has demonstrated its release from the myocardium⁽²¹²⁾ and that infusion of ADM in HF patients results in significant vasodilatation, increase in cardiac index and reduction of pulmonary capillary wedge pressure⁽²¹³⁾.

In addition to its vasoactive properties, ADM also demonstrates neurohormonal effects (figure 16). Several studies have demonstrated that ADM inhibits protein synthesis and hypertrophy of cardiomyocytes, as well as proliferation of cardiac fibroblast and production of extracellular matrix⁽²¹⁴⁾. In human subjects, it has been shown to increase plasma renin concentrations⁽²¹⁵⁾ but selectively inhibited the aldosterone response to the pressor effects of Ang II⁽²¹⁶⁾. Furthermore, infusions of ADM in higher doses than those observed in pathophysiological states have been shown to increase renal blood flow, urine output, and sodium excretion in a NO dependent manner⁽²¹⁷⁾.

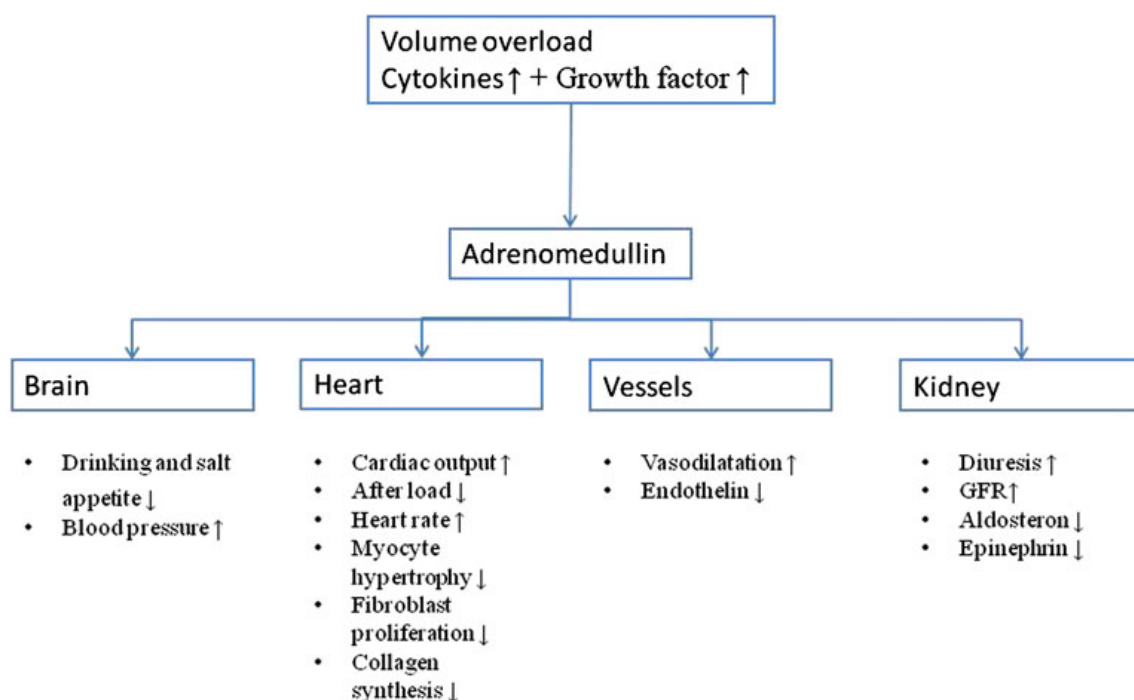


Figure 16: Actions of adrenomedullin in acute heart failure⁽²¹⁸⁾

1.4.2 Adrenomedullin for diagnosis of heart failure

Early human studies confirmed increased plasma concentrations in severe cardiac failure compared with healthy controls⁽²¹⁹⁾ and correlation with decreasing LVEF⁽²²⁰⁾. Studies have attempted to evaluate the potential of MRproADM for the diagnosis of HF in patients presenting with dyspnoea but to date, the ability of MRproADM to accurately diagnose the condition remains inferior to that of the natriuretic peptides^(221, 222). The main role of MRproADM appears to lie in its ability to predict prognosis in both acute and chronic HF.

1.4.3 Adrenomedullin for prognosis of heart failure

In the Biomarkers in ACute Heart failure (BACH) trial⁽²²³⁾, MRproADM was powerfully prognostic for death at ninety days in patients presenting with acute HF, adding value above that of BNP. Studies have also confirmed MRproADM to be strongly correlated with disease severity and as a predictor of mortality in chronic HF patients independent of other established prognostic factors including NTproBNP, LVEF, NYHA class, creatinine and age⁽²²⁴⁾. More recently, Adlbrecht et al⁽²²⁵⁾ showed that the prognostic value of BNP was, in fact, inferior to that of MRproADM in a cohort of over seven hundred outpatients with chronic HF. Moreover, the Australia-New Zealand Heart Failure Study demonstrated that patients treated with carvedilol had reduced risk of death or HF hospitalisations in patients with above median levels of MRproADM⁽²²⁶⁾.

1.4.4 Adrenomedullin for monitoring of heart failure

To date, only one published study has attempted to evaluate the use of serial measurements of MRproADM. In this, Neuhold et al⁽²²⁷⁾ studied 181 patients with a recent HF hospitalisation undergoing optimisation of prognostically indicated medications. Their results indicated that although absolute baseline and follow-up concentrations of BNP and MRproADM were equally predictive of all-cause mortality, relative changes added no prognostic information in this patient cohort. Unfortunately, almost half of the hospitalisations recorded in this study occurred prior to the

three-month study follow-up and therefore also prior to full optimisation. No studies have evaluated the use of serial monitoring in pharmacologically stable chronic HF patients.

1.4.5 Biological variability of adrenomedullin

One study has examined the biological variability of MRproADM in disease. In a study of 52 patients undergoing orthotopic liver transplantation, Miguel et al⁽²²⁸⁾ calculated the one week biological variability of MRproADM in a sub-set of patients with an uncomplicated post-operative course. Their results found a reference change value of 112% in this cohort of patients. No data currently exists on the biological variability of ADM or MRproADM in the HF setting.

1.5. Apelin

Apelin is an endogenous vasodilatory peptide with inotropic properties and is the only known ligand for the apelin (APJ) receptor. It shares similarities with the Ang II type 1 receptor pathway. Both apelin and its receptor are widely distributed in the vascular endothelium of many organs, including the heart. Studies in both animals and humans have suggested it may play a role in the pathogenesis of HF by modulating the harmful effects of Ang II.

1.5.1 Apelin release and cardiovascular effects

First identified in 1992, the APJ receptor was noted to have marked similarities to the angiotensin I receptor but, at the time, no known ligand⁽²²⁹⁾. It remained an “orphan receptor” until 1998 when apelin was isolated from bovine stomach extracts⁽²³⁰⁾.

Apelin is secreted as a 77-amino acid pre-proprotein. After translocation into the endoplasmic reticulum and cleavage of the signal peptide, the proprotein of 55 amino acids may generate several active fragments: a 36-amino acid peptide corresponding to the sequence 42-77 (apelin 36), a 17-amino acid peptide corresponding to the sequence 61-77 (apelin 17) a 13-amino acid peptide corresponding to the sequence 65-77 (apelin 13) and a conserved 12 amino acid C-terminal peptide (apelin-12). Both apelin and APJ messenger RNA (mRNA) is expressed extensively in body tissue, with high concentrations of the APJ receptor found in cardiac tissue⁽²³¹⁾.

The expression of apelin and APJ is regulated by multiple factors, including hypoxia⁽²³²⁾, TNF- α ⁽²³³⁾, Ang II⁽²³⁴⁾ and some bone morphogenic proteins⁽²³⁵⁾. In animal models of HF, apelin mRNA was markedly down regulated in rat myocytes subjected to stretch as well as in two further models of chronic ventricular pressure overload⁽²³⁶⁾. This finding has been further corroborated in human subjects, with reduced concentrations of peripheral plasma apelin in those with HF compared with those without structural heart disease. Moreover, levels of apelin were found to increase in patients

who exhibited left ventricular reverse remodelling and an increase in ejection fraction post CRT. Furthermore, in murine models of hypertensive heart disease, the expression of cardiac apelin and APJ shows no change at the stage of compensatory left ventricular hypertrophy, but is markedly decreased at the stage of HF⁽²³⁷⁾.

The actions of apelin and the APJ receptor trigger a variety of cellular signaling pathways. In the cardiovascular system, these pathways are involved in a number of physiological actions (figure 17).

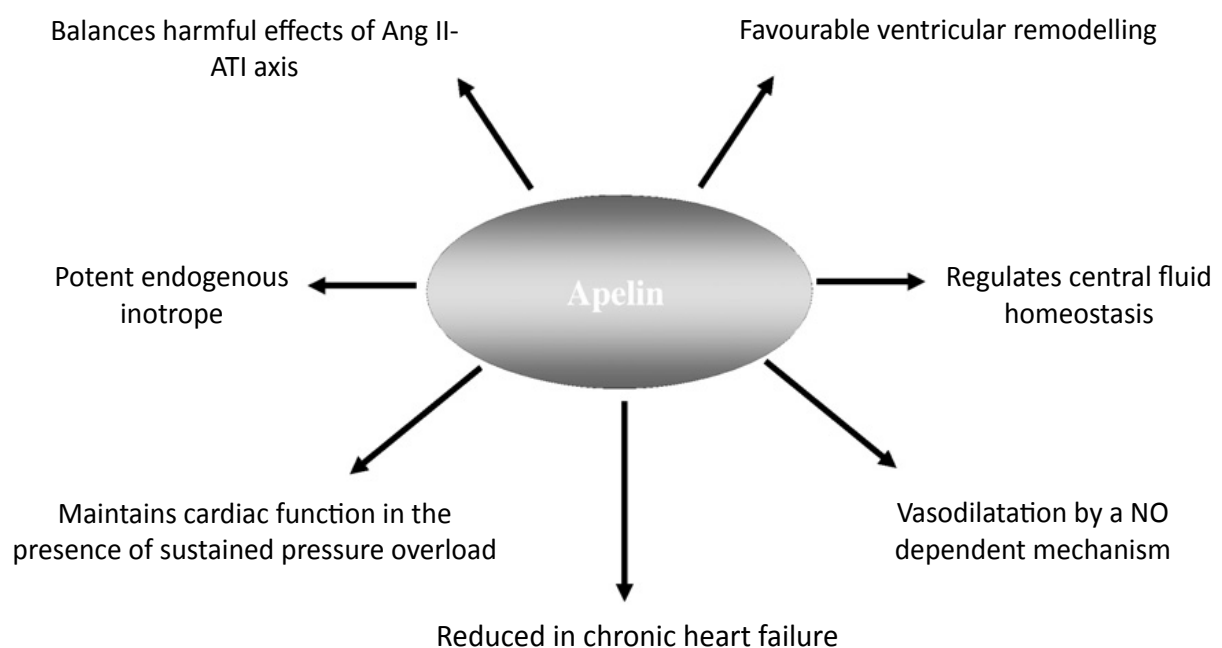


Figure 17: Proposed role of apelin in heart failure (adapted from⁽²³⁸⁾)

A potent inotropic effect has been demonstrated *in vivo* and *ex vivo* in normal rat and mice hearts during apelin infusion of varying durations^(236, 239-241). The exact mechanism by which apelin exerts its inotropic effects remains unknown however this is believed to be via an increase in intracellular calcium (figure 18)⁽²³⁸⁾. This proposal is supported by the finding that apelin induced inotropy is attenuated by inhibition of phospholipase C, protein kinase C and the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ and Na^+/H^+ exchangers⁽²⁴⁰⁾.

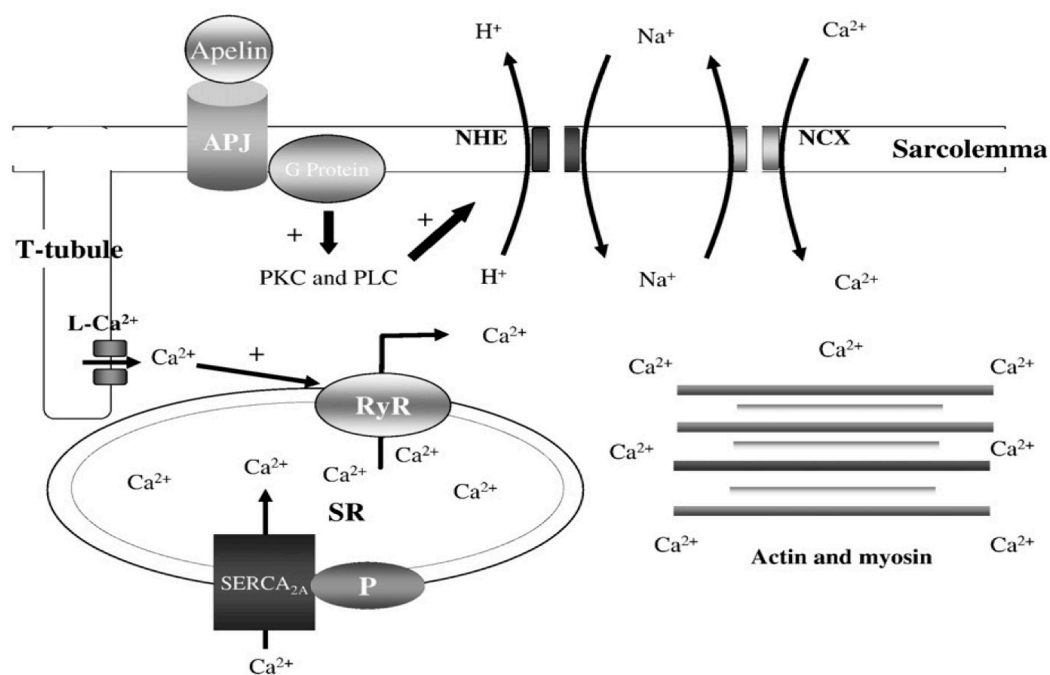


Figure 18: Proposed actions of apelin in cardiac myocyte contraction⁽²³⁸⁾

Calcium enters the cell via L-type calcium channels ($L\text{-Ca}^{2+}$) and initiates calcium release from the sarcoplasmic reticulum (SR) by activating the ryanodine receptor (RyR). This massive Ca^{2+} release is required to trigger the contractile process. Calcium reuptake is mediated by the energy requiring sarcoendoplasmic reticulum Ca^{2+} -adenosine triphosphatase (SERCA_{2A}) and Phospholamban (P), which in turn allows relaxation. Apelin activates protein kinase C (PKC) and phospholipase C (PLC) that acts on the sarcolemmal, $\text{Na}^{+}\text{-H}^{+}$ exchanger (NHE) to increase intracellular Na^{+} resulting in an increase in intracellular Ca^{2+} by action of the reverse mode $\text{Na}^{+}\text{-Ca}^{2+}$ exchanger (NCX). This leads to an increase in available cytosolic calcium for the cardiac contractile apparatus.

Via the action of endothelial nitric oxide, apelin also causes peripheral vasodilatation and has been shown to induce diuresis by counteracting the actions of arginine vasopressin⁽²³⁸⁾. Furthermore, injection of apelin into peri-infarct areas of ischaemic myocardium facilitates neovascularization⁽²⁴²⁾. These observed effects and proposed roles of apelin have raised the possibility that the apelin-APJ pathway may represent a novel 'compensatory' endogenous system in HF and therefore not only a novel biomarker but a future therapeutic target.

1.5.2 Apelin for diagnosis of heart failure

Studies looking at the use of apelin to aid diagnosis of HF have, to date, been disappointing. In patients presenting to the emergency department with acute dyspnoea, apelin was not able to predict the presence of acute HF⁽²⁴³⁾. Furthermore, compared with NTproBNP, N-terminal pro-atrial natriuretic peptide (NTproANP), interleukin-6, TNF- α and both adrenaline and noradrenaline, apelin

concentrations were unable to distinguish patients with known idiopathic dilated cardiomyopathy from a control group and had no relationship to the level of cardiac dysfunction⁽²⁴⁴⁾. Evidence from one study indicates that apelin may be reduced in all forms of cardiopulmonary disease, thereby limiting both its sensitivity and specificity in the HF setting⁽²⁴⁵⁾.

1.5.3 Apelin for prognosis in heart failure

Few published studies have compared the prognostic ability of apelin with that of the B type natriuretic peptides or other biomarkers^(243, 246). At present, such studies have indicated apelin to be inferior in this respect.

Although there is little in the way of studies looking at the response of apelin to medical therapy, there are encouraging reports of response with reverse remodelling following device therapy. In one study of patients with end-stage HF, tissue concentrations of apelin were increased and the APJ gene was up-regulated following left ventricular assist device implantation and successful left ventricular reverse remodelling⁽²⁴⁷⁾. Furthermore, plasma apelin has been shown to increase in patients with significant reverse remodelling following CRT⁽²⁴⁸⁾. There are no published longitudinal data on how apelin levels change over time in stable HF patients.

1.5.4 Apelin for monitoring heart failure

To date, no studies have evaluated the potential use of apelin for monitoring patients with chronic stable HF.

1.5.5 Biological variability of apelin

To date, no data exists on the biological variability of apelin, either in health or disease states.

1.6. ST2

Suppression of Tumorigenicity (ST2) was originally identified as a member of the IL-1 receptor family in 1989⁽²⁴⁹⁾. It exists in two distinct forms; a transmembrane form – the ST2 ligand (ST2L), and a soluble, circulating form (sST2). These isoforms result from alternative splicing and 3' processing of the ST2 gene at the RNA level. sST2 is a variant of the full-length gene, which lacks the transmembrane and cytoplasmic domains contained within the structure of the ST2L isoform. With no obvious functional endogenous ligand, for many years it was considered an 'orphan' receptor that appeared to have a mediatory role in inflammatory conditions via the action of the Th2 subset of T-helper cells and the production of Th2-associated cytokines⁽²⁵⁰⁾. A cardiovascular role for ST2 was not suggested until 2002 when Weinberg et al, demonstrated up-regulation of both isoforms of ST2 by mechanically stressed cardiomyocytes and fibroblasts in an in vitro model⁽²⁵¹⁾. It was not until 2005, however, that the discovery of interleukin-33 (IL-33) as an ST2 ligand provided new insights into ST2 signalling⁽²⁵²⁾.

1.6.1 ST2 Release and cardiovascular effects

Subsequent work looking at the role of ST2 in cardiovascular disease led to the proposal that the IL-33/ST2L pathway works as part of a cardioprotective signalling system that regulates responses of the ventricular myocardium to altered loading conditions, including myocyte hypertrophy and fibrosis⁽²⁵³⁾. One possible mechanism by which ST2 may exert its effects was proposed by Seki et al, who demonstrated decreased caspase-3 activation, a critical step in apoptosis, and increased expression of the anti-apoptotic gene Bcl-2 in rat cardiomyocytes exposed to IL-33 in vitro. Such proposals have, to date, yet to be fully established. Despite this, however, derangement of the signalling system has been consistently demonstrated to result in a phenotype consistent with myocardial remodelling in patients with chronic HF, supporting its proposed pathophysiological role. In an in vivo model of ventricular pressure overload, mice subjected to partial aortic constriction were shown to have enhanced IL-33 protein expression in the fibroblasts of the left ventricle.

Furthermore, the same mice treated with exogenous IL-33 demonstrate reduced hypertrophy; whilst those bred with transgenic deletion of ST2L demonstrate abolishment of this salutary effect, resulting in severe myocardial fibrosis and hypertrophy⁽²⁵³⁾.

Whereas ST2L has been demonstrated to mediate the effects of IL-33, research looking at sST2 has implicated it in having an opposing, attenuating role against the actions of the IL-33/ST2L pathway. In this way, it is proposed to act as a 'decoy' receptor. Indeed, in the face of exogenous pro-hypertrophic stimuli such as Ang II, IL-33 administration to cultured rat cardiomyocytes results in attenuation of cellular hypertrophy. If sST2 is added to the cell culture media, however, these effects of IL-33 are not only abolished, but reversed⁽²⁵³⁾. It is thought, therefore, that sST2 in the extracellular environment acts by binding free IL-33, thereby effectively decreasing the concentration of IL-33 that is available for ST2L binding, reducing the biological effect of IL-33 and modulating IL-33/ST2L signalling.

It is the soluble form, sST2, to which assays are available. Currently, however, there are only a limited number of enzyme-linked immunosorbent assays (ELISA) for the detection and measurement of sST2. Early assays suffered from low sensitivity and poor analytical coefficient of variation (CV_a). More recently, the 'Presage ST2®', a highly sensitive ELISA for sST2 has been developed and validated in a large cohort⁽²⁵⁴⁾. Interestingly, although ST2 is associated with allergic and immunologic diseases such as asthma, among subjects with normal heart function, sST2 was not found to be higher in those with such underlying conditions. In addition, unlike the natriuretic peptides, sST2 concentrations were not significantly affected by obesity, age, atrial fibrillation, aetiology of cardiomyopathy, or prior diagnosis of HF.

1.6.2 ST2 for diagnosis of heart failure

The first large-scale analysis of sST2 in patients with chronic HF was from the Pro-BNP Investigation

of Dyspnea in the Emergency Department (PRIDE) study⁽²⁵⁵⁾. In this study looking at 593 patients admitted to the emergency department with acute dyspnoea, sST2 concentrations were higher in those with HF than those with non-cardiac breathlessness. Moreover, there was a concentration-dependent relationship between sST2 and risk of death at one year, with its prognostic value being additive to that of NTproBNP. Compared with NTproBNP, however, sST2 was less accurate as a diagnostic biomarker for HF with area under curve (AUC) 0.94 vs. 0.74 respectively⁽¹⁶⁹⁾.

1.6.3 ST2 for prognosis of heart failure

Experiments looking at serum sST2 following the induction of myocardial infarction first indicated the potential of sST2 as a biological marker for mechanical overload in the heart. In patients presenting with ST-elevation myocardial infarction (STEMI), sST2 levels at the time of presentation correlated with the incidence of in-hospital and thirty-day mortality⁽²⁵⁶⁾. Given that mechanical overload of the myocardium is a feature of many types of HF, Weinberg et al further demonstrated that sST2 levels in patients with non-ischaemic HF not only correlated with serum BNP levels but also independently predicted risk of reaching a subsequent end-point of mortality or cardiac transplantation⁽²⁵⁷⁾. The benefits of sST2 as a prognostic marker in acute/recently-decompensated HF are now well documented^(138, 258-261). Far fewer studies, however, have examined the role of sST2 in patients with chronic HF.

Ky *et al*⁽²⁶²⁾, in a study of 1141 outpatients with a clinical diagnosis of HF demonstrated an independent association between single baseline measurements of sST2 and risk of adverse outcomes. Gruson *et al*⁽²⁶³⁾ have reported similar findings with an association between baseline sST2 and CV mortality in a sample of 137 outpatients with reduced LVEF treated with optimal therapy. More recently, Sobczak *et al*⁽²⁶⁴⁾ have demonstrated the ability of single baseline sST2 to predict adverse outcome at one-year in 145 patients who were clinically stable for four weeks with LVEF<30% and treated with maximum tolerated doses of guideline based medication.

Overall, sST2 appears to be a strong prognostic marker in both acute and chronic HF, making it an attractive biomarker for monitoring potential.

1.6.4 ST2 for monitoring heart failure

The use of serial monitoring of sST2 has been of increasing interest over recent years. Four studies have published data involving serial sST2 measurements in patients with acute HF^(258-260, 265). In 2008, Boisot et al⁽²⁵⁹⁾ published data from 150 patients in whom samples were collected on admission and discharge as well as up to four consecutive days during admission. In this study, percentage change in sST2 was equal to baseline NTproBNP for predicting 90-day mortality. Bayes-Genis et al⁽²⁶⁵⁾ further corroborated these findings in 2010, by demonstrating that percentage change in sST2 over a two-week sampling period was predictive of cardiac events (including death, HF admission and heart transplantation) at one year. More recently, however, Manzano-Fernandez et al⁽²⁶⁰⁾, in a study of seventy-two acute HF patients, found that sST2 concentrations at presentation had a greater prognostic accuracy for two-year mortality than percentage change in sST2 between baseline and four days. In the largest series to date, Breidthardt et al⁽²⁵⁸⁾ examined changes in sST2 concentrations at baseline and at forty-eight hours in 207 patients with acute HF. Baseline sST2 concentrations significantly predicted both in-hospital and one-year mortality. Similarly, percentage change in sST2 over the 48hr period was equally as predictive.

There are limited data on the use of sST2 in chronic HF. In their study of non-ischaemic HF patients with NYHA class III-IV, Weinberg et al suggested that changes in sST2 over a two-week period, but not baseline sST2 concentrations, were associated with an increased risk of death or transplantation at one year⁽²⁵⁷⁾.

1.6.5 Biological variability of ST2

Only one published study has explored the biological variability of sST2. In this study, Wu et al⁽²⁶⁶⁾

revealed the RCV of sST2 in healthy volunteers to be 30% - much lower than that of the B-type natriuretic peptides. In support of this finding, Breidhardt⁽²⁶⁷⁾ reported a decrease in sST2 greater than 50%, in response to the initiation of HF therapy, was associated with a significantly less one year mortality rate than in those with less than a 50% decrease in sST2 (11% vs 41% respectively).

Despite such promising results, it is likely that variation amongst patients with disease reflects the continuous stages of disease variation, and as such, is likely to be greater than that in healthy individuals. To date, no studies on the biological variability or RCV of sST2 in the disease state have been reported.

1.7. Galectin-3

Galectin-3 is a soluble β -galactosidase-binding lectin implicated in the regulation of cardiac fibrosis and remodelling. It is a 29-35kDa chimaera-type galectin, unique for its presence of an extended N-terminal domain linked to a single C-terminal carbohydrate-recognition domain. Galectin-3 is found in a wide range of tissues and is localised primarily in the cytoplasm and occasionally in the nucleus and mitochondria. When secreted into the extracellular space, it can interact with cell surface receptors and glycoproteins to initiate transmembrane signalling pathways for different cellular functions.

1.7.1 Galectin-3 release and cardiovascular effects

In normal human hearts, the expression of galectin-3 is low⁽²⁶⁸⁾. In the disease state, however, there is rapid up-regulation. Although the exact myocardial localisation of galectin-3 has yet to be definitively established, immunochemistry of hypertrophied rat myocardium has demonstrated binding-sites localised predominately in the myocardial matrix, fibroblasts and macrophages⁽²⁶⁸⁾. Moreover, in proliferating fibroblasts of the same hypertrophied hearts, these binding-sites were localised around the nucleus, with only minimal cytoplasmic binding sites observed in resting fibroblasts, suggesting that galectin-3 may induce cardiac fibroblast proliferation⁽²⁶⁸⁾. Furthermore, during active myocarditis, galectin-3 was found to be co-localised with activated macrophages, suggesting galectin-3 is not only released by activated macrophages, but may also have a stimulatory effect for macrophage migration.

Fibroblasts and macrophages are key cells in the initiation and progression of fibrosis and tissue scarring. Up-regulation of galectin-3 has been demonstrated in animal models of hepatic, renal and cardiac fibrosis and subsequently observed in human fibrotic conditions such as liver cirrhosis⁽²⁶⁹⁾, chronic pancreatitis⁽²⁷⁰⁾ and pulmonary fibrosis⁽²⁷¹⁾. Moreover, galectin-3 has been shown to be both temporally and spatially associated with fibrosis in rat liver, being minimal in the normal liver,

maximal at peak fibrosis and then absent after recovery⁽²⁷²⁾.

Thus, although there are strong arguments connecting galectin-3 with cardiac remodelling, to date there is no definitive evidence that a causal relationship exists between galectin-3 and the pathophysiology of cardiac remodelling. Further work continues in this field to fully establish this link.

1.7.2 Galectin-3 for diagnosis of heart failure

Several studies have examined the use of galectin-3 as a biomarker for HF diagnosis^(243, 273, 274). To date, such studies have indicated that galectin-3 is inferior to the natriuretic peptides for this purpose. Using data from the Pro-BNP Investigation of Dyspnea in the Emergency Department (PRIDE) study⁽¹⁶⁹⁾, van Kimmenade et al demonstrated that NTproBNP was significantly better than galectin-3 for the diagnosis of HF (AUC 0.94 vs 0.72 respectively; $p < 0.0001$)⁽²⁴³⁾. More recently, Mueller et al have corroborated these findings, demonstrating an AUC of 0.92 for BNP compared with 0.57 for galectin-3 for the diagnosis of HF in 251 patients presenting to the emergency department with acute dyspnoea⁽²⁷⁴⁾. Moreover, the addition of galectin-3 to BNP did not add to the diagnostic capability of BNP, nor were increased levels of galectin-3, defined as $>25.9\text{ng/ml}$, independently associated with the diagnosis of acute HF⁽²⁷⁴⁾.

One possible explanation for these findings relates to the proposed relationship of galectin-3 to fibrosis and scarring. Given that these phenomena are presumed to be relatively late manifestations of cardiac dysfunction and are therefore more likely to be associated with chronic disease progression, rather than acute decompensation. Conversely, as a measure of stretch, the natriuretic peptides are more likely to be elevated in circumstances of both pressure and volume overload, both of which have been demonstrated in the acute HF setting⁽¹⁾. Clinically, the role of galectin-3 appears to be in its ability for prognostication.

1.7.3 Galectin-3 for prognosis of heart failure

Numerous studies have examined the prognostic value of galectin-3 in both acute and chronic HF^(243, 275-280). Such studies have demonstrated that galectin-3 levels are strongly related to outcome and are independent predictors of mortality in a wide range of clinical presentations, including those in the community with incident HF⁽²⁷⁷⁾, acute HF⁽²⁴³⁾ and those with end-stage HF requiring mechanical support⁽²⁸¹⁾. Furthermore, in their study of acutely dyspnoeic patients presenting to the emergency department, Shah et al⁽²⁸⁰⁾ demonstrated that, although galectin-3 levels were not associated with echocardiographic markers of left ventricular systolic dysfunction, they were related to Doppler indices of higher filling pressures and right ventricular systolic dysfunction. Consequently, galectin-3 concentrations remained an independent predictor of mortality for up to four years.

1.7.4 Galectin-3 for monitoring heart failure

Several studies have examined the use of serial galectin-3 measurements in HF patients^(136, 137, 282). In a study by Motiwala et al⁽¹³⁶⁾, using patients enrolled in the PROTECT trial, additional galectin-3 sampling at six months provided significantly greater prognostic information than that of the baseline level alone. Moreover, they demonstrated that an increase of $\geq 15\%$ at any of the three-monthly review time points conferred worse prognosis, even after extensive clinical adjustments. This finding was also noted by van der Velde and colleagues⁽¹³⁷⁾. Using the Val-HEFT population, Anand et al⁽²⁸²⁾ showed that, over a four-month follow-up, for every 1ng/ml increase in galectin-3, there was an associated 2.9% increase in risk of mortality ($p=0.008$) and a 2.1% increased risk of first morbid event ($p=0.03$). To date, however, no studies have been able to demonstrate any effect on galectin-3 levels with the use of prognostically indicated medications, potentially limiting its usefulness for monitoring purposes.

1.7.5 Biological variability of galectin-3

To date, only two published studies have explored the biological variability of galectin-3 in human

subjects^(266, 283). In a study on seventeen healthy subjects, Wu et al⁽²⁶⁶⁾ reported intra-individual coefficient of variation of galectin-3 of 16% at one hour and 20% at two months. This resulted in computed reference change values of 39% and 61% respectively, significantly lower than that of the natriuretic peptides, but higher than that of sST2. More recently, Franekova et al⁽²⁸³⁾ have reported on a series of 44 patients followed for 12 months post heart transplant. In this study, the authors report a one-year intra-individual coefficient of variation of 28.2%, giving an overall RCV of 78.6%. Using their results RCVs for both increase and decrease in galectin-3 concentrations were also calculated at 116%, and –53.7%, respectively, leading the authors to conclude that the concentration of galectin-3 would need to approximately double or decrease by half to indicate a new process. Such findings have further put the potential usefulness of galectin-3 as a monitoring tool in doubt.

RESEARCH OBJECTIVES

The aim of this MD is to study the monitoring potential of 4 novel biomarkers for chronic HF: Mid-Regional pro-adrenomedullin (MRproADM), Apelin, soluble ST2 (sST2) and Galectin-3.

To achieve this I will:

1. Measure the biological variability in patients with stable chronic HF at several time points: one hour, one month, three months and six months and compare with NTproBNP in the same cohort.
2. Calculate the reference change values at one hour, one month, three and six months and compare these with the RCV of NTproBNP in the same cohort.
3. Perform ROC analyses to assess the ability of the novel biomarkers to predict HF decompensation.
4. Perform a multi-marker comparison to assess the added value of combining markers in predicting outcome in chronic HF.

By assessing the putative value of these biomarkers, my hypothesis is that one or more of these novel biomarkers will have a RCV lower than that of NTproBNP. In addition, it is hypothesised that the percentage and/or absolute changes over time will be better than that of NTproBNP in predicting HF decompensation, defined as a CV admission during the study period. This being so, it would then provide evidence to justify a randomised controlled trial (RCT) of therapy monitoring in chronic HF using one or more of these novel biomarkers.

MATERIALS AND METHODS

3.1 Study population

Between October 2011 and April 2013, 50 patients with chronic HF NYHA Class I-III and LVEF $\leq 40\%$ were recruited from the HF clinics at King's College Hospital, London. All patients were on optimum tolerated medications, comprising of ACE-I or ARB, β -blockers, MRA and diuretics. Target dose levels were defined per the 2012 ESC guidelines⁽²²⁾. Main exclusion criteria were a CV admission or change in prognostically indicated medication within four weeks of recruitment, significant renal impairment (estimated glomerular filtration rate (eGFR) <20 mL/min/1.73 m²), or the inability or unwillingness to consent.

Recruitment took place over a 2.5-year period with over 4000 patients seen in both physician and HF nurse led clinics and screening of approximately 350 patients. All patients were enrolled prior to the publication of the PARADIGM-HF trial. Consequently, no patients were treated with sacubitril-valsartan.

Clinical review and blood sampling took place at five time points – baseline, one hour, one month, three months and six months. Patient reviews took place at the same time of day for each visit. The same cardiologist reviewed and performed all venepuncture at each time point. Vital signs and NYHA class were recorded together with medications and details of any hospital admissions. The reviewer was blinded to the results of any previous biomarker results.

The primary endpoint of the study was CV admission, defined as an admission due to decompensated HF, arrhythmia or acute coronary syndrome. Justification for this endpoint is based on previous work (as cited in section 1). Given the small sample size, a composite endpoint was deemed most suitable to capture sufficient events for statistical analysis. Associations with ADHF, fibrosis and myocardial ischaemia demonstrated for the novel biomarkers in question led to the conclusion that, assuming fibrosis may be associated with arrhythmia, these events would be most

likely to be represented by changes in the biomarkers of interest.

3.2 New York Heart Association (NYHA) classification

The NYHA classification is used to describe the functional status of HF patients based on symptomology and levels of physical activity. It has previously been shown to correlate with degree of LVSD and prognosis⁽²⁸⁴⁻²⁸⁷⁾.

Class I	Few observable symptoms. No limitation in ordinary physical activity
Class II	Mild observable symptoms. Slight limitation in physical activity – symptoms during ordinary physical activity.
Class III	Moderate observable symptoms. Marked limitation in physical activity - symptoms during less-than-ordinary activity.
Class IV	Severe observable symptoms. Severe limitation in physical activity - symptoms at rest.

3.3 12 Lead ECG

A 12-lead electrocardiogram (ECG) was performed in all patients at baseline and six-month visits. The cardiac investigation department at King's College Hospital performed all ECGs using a GE Mac 5500 machine. Standard paper speed of 25mm/s was used.

3.4 Echocardiography

All study participants had undergone transthoracic echocardiography (TTE) within six months of recruitment. The echocardiography department at King's College Hospital performed all scans with iE33 echocardiography machines (Philips Medical Systems, Andover, Massachusetts). Images obtained were analyzed using dedicated image-viewing software (Xcelera R3.2L1 SP2 version,

Phillips). Each study was performed and reported in accordance with the European Association of Echocardiography guidelines. Where imaging quality was sufficient, LVEF was measured by the quantitative two-dimensional (biplane Simpson) method. In patients with poor echo windows, LVEF was estimated visually by the same cardiologist to reduce inter-operator error.

3.5 Biochemical analysis

Blood samples were obtained by venepuncture after 30 minutes of semi-recumbent rest. Two x 10 mL of venous blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes and one x 10 mL of venous blood was drawn into a bottle without anti-coagulant. Serum samples for creatinine were analysed immediately. After centrifugation at 3000 rpm, 2 mL aliquots of plasma were stored at -30°C until analysed.

3.5.1 NTproBNP

NTproBNP was measured by two-site chemiluminescence immunoassay (Immulite 2000, Siemens Healthcare Diagnostics Ltd, Camberley, Surrey UK). The intra-assay precision was 5.4, 3.0 and 4.1% and the inter-assay precision was 6.4, 4.0 and 4.7% at 35.6, 1430 and 29725 ng/L respectively. The limit of detection was 10 ng/L. Reference concentrations for this assay are 125 ng/L and 450 ng/L at age <75 and >75 respectively.

3.5.2 Mid-Regional pro-Adrenomedullin

MRproADM analysis was performed using a novel automated sandwich chemiluminescence immunoassay (BRAHMS), which utilises a purified sheep polyclonal preproADM specific (amino acids 83-94) antibody and a sheep polyclonal preproADM specific (amino acids 68-86) antibody labelled with MACN-acridinium-NHS-ester. This method has previously been described in detail and has been validated in previous studies^(221, 224, 288). From the data available to date, the following reference table (table 7) can be used for the assessment of MRproADM concentrations in healthy subjects.

Normal subjects	MRproADM nmol/L
97.5 % percentile	0.55 nmol/L
Median	0.39 nmol/L

Table 7: Reference concentrations for MRproADM in normal subjects

Based on recent publications a calculated cut off >1.5 nmol/L indicates an individual high risk concerning severity of disease and prognosis⁽²²³⁻²²⁵⁾. For the purpose of this study, concentrations above 0.55 nmol/L were considered above the normal reference range.

3.5.3 Apelin

Concentrations of apelin were determined using a commercially available enzyme immunoassay (EIA) without extraction (Phoenix Pharmaceuticals), the use of which has been described previously⁽²⁸⁹⁻²⁹²⁾. This assay uses an immunoaffinity purified rabbit antibody specific to apelin-12 which recognises all biologically active isoforms of apelin (100% cross-reactivity to human apelin-12, -13, and -36) but has no cross reactivity to adrenomedullin-52, BNP-32, CNP-22, ANP (25-56), ghrelin, endothelin-1 or bradykinin. The current quoted reference range for apelin 12 is 580 – 1680 ng/L.

3.5.4 Soluble ST2

sST2 was measured by enzyme linked immunosorbent assay (ELISA) (R&D Systems Europe, Ltd., Abingdon, UK). The sST2 assay contains NS0-expressed recombinant human sST2 and has been shown to accurately quantitate the recombinant factor. The intra-assay precision was 5.6, 4.4 and 4.5% and the inter-assay precision was 7.1, 5.4 and 6.3% at 5.4, 12.6 and 20.6 µg/L respectively. The limit of detection was 0.005 µg/L. Reference range is 6.74 – 20.4 µg/L.

3.5.5 Galectin-3

Plasma galectin-3 concentrations were determined using a quantitative sandwich enzyme

immunoassay containing an *E-coli* expressed recombinant human galectin-3 (Quantikine Galectin-3 ELISA kit distributed by R & D Systems Europe, 19 Barton Lane, Abingdon Science Park, Abingdon, Oxon, OX14 3NB). This assay has been shown to accurately quantitate the recombinant factor. The intra-assay precision was 4.3, 3.8 and 3.5% at 0.79, 2.46 and 5.11 µg/L respectively. The inter-assay precision was 5.8, 6.0 and 5.8% at 0.80, 2.59 and 5.50 µg/L respectively. The minimal detectable concentration is 0.016 µg/L. The reference range is 2.40 – 15.7 µg/L.

3.5.6 Creatinine

Creatinine was measured using the Jaffé reaction using reagents supplied by Siemens Healthcare Diagnostics Ltd, Camberley, Surrey, UK. The initial rate of absorbance change is measured at a wavelength of 505 nm and compared to that of a known calibrant. This is directly proportional to the concentration of creatinine in the sample. A blank reaction rate is performed using reagent 1 (sodium hydroxide – before picrate addition) to minimise interference from bilirubin. Reference range is 45-120 µmol/l.

3.5.7 Estimated Glomerular Filtration Rate (eGFR)

Estimated glomerular filtration rate was calculated using the abbreviated (four-variable) Modification of Diet in Renal Disease (MDRD) equation in accordance with guidelines of the National Kidney Disease Education Program and the Renal Association^(293, 294).

3.5.8 Sodium (Na)

Serum electrolytes were measured using the Siemens Advia 2400 analyser with ion selective electrode (ISE) reagent supplied by Siemens Healthcare Diagnostics Ltd, Camberley, Surrey, UK.

The serum sample is mixed with ISE buffer (1:33 dilution) to provide a constant pH and a constant ionic strength solution. As the buffered sample flows past the electrode a new equilibrium forms due to selective ion transport at the electrode surface and creating a change in measured potential

difference in the cell. This electrical potential is measured against a reference electrode and is logarithmically related (via the Nernst equation) to the sodium, potassium or chloride concentration in the sample. The quoted analytical ranges for sodium are 100-200mmol/L with a reference range of 135 - 145 mmol/L.

3.5.9 Haemoglobin (Hb)

The Siemens Advia 2120i was used to analyse the full blood count (FBC) using reagents supplied by Siemens Medical Solutions Diagnostics Limited, Newbery, Berks, UK. Haemoglobin measurements were calculated using cyanide free haemoglobin detection (HGB).

There are 2 methods employed for this form of quantification:

Method 1: a cyanide free colourimetric method, which lyses the RBC to release the haemoglobin. The haem ion is oxidised to the ferric state where it combines with the reagent to form an axial ligand monoaquomonohydroxyferri-porphyrin. Optical readings are taken at a wavelength of 456nm and plotted on the haemoglobin transmission histogram. This method is affected by background colouration e.g. lipaemia, haemolysis and icteric samples.

Method 2: Cellular HGB is a calculated method which uses the directly measured parameter CHCM (Corpuscular Haemoglobin Concentration Mean) it is calculated using the following values $\text{CHCM} \times \text{RBC} \times \text{MCV} / 1000$. This method is not affected by either haemolysed, lipaemic or icteric samples.

The 2 methods should not differ by more than 1.9g/dl however if they do an error message is flagged. The CHCM is only used when the haemoglobin is falsely increased and the MCHC is also falsely increased. Using CHCM to calculate Cellular HGB avoids this interference since CHCM is directly measured and is virtually unaffected by lipaemia.

3.5.10 Bilirubin

Total bilirubin was calculated using the Siemens Advia 2400 assay using reagents supplied by Siemens Healthcare Diagnostics Ltd, Camberley, Surrey, UK. The principle of the assay is based on the fact that bilirubin reacts under acidic conditions with vanadate ions, resulting in its oxidation to biliverdin. In the presence of detergent and a citrate buffer, both conjugated and unconjugated bilirubin are oxidised. The decrease in bilirubin concentration which results from this is measured at 451 nm, and the difference in absorbance over the five-minute reaction period is related to bilirubin concentration by comparison to a previous calibration assay. The concentration is then measured as an endpoint reaction. The assay is linear to 598 µmol/L. The reference range is 3 - 20 µmol/L.

3.6 Statistical analysis

3.6.1 Sample size/Power calculation

The initial hypothesis of this study was aimed at examining the biological variability of novel biomarkers compared with the natriuretic peptides. As there had been no longitudinal studies which had measured concentrations of these novel peptides, it was not possible to accurately power the study for a certain percentage reduction in RCV compared with the RCV of BNP. However, previous work on the biological variability of BNP and NTproBNP in both healthy controls and patients with LVSD were able to generate meaningful data from cohorts of up to 45 patients^(188-190, 295). Using such data, the sample size of 50 was chosen as a reasonable estimate.

Whilst this sample size was likely to be insufficient for firm conclusions regarding monitoring, it was hoped that estimates provided would be valuable to help power a larger trial looking at this aspect of the study.

3.6.2 Descriptive Statistics

Categorical variables are described as proportions. Continuous variables are described with mean

and standard deviation for normally distributed variables and median and interquartile range (IQR) for non-normally distributed variables. The Shapiro-Wilk test was used to assess the data for distribution normality.

Changes in biomarker concentrations are assessed in terms of both absolute and percentage change relative to the baseline for each patient. Justification for using this method of evaluation, rather than categorical cut-off values, is based on both the stability of the patients, the small sample size of the study and an attempt to examine a more individualised method of monitoring using biological variability as a guide of meaningful change.

Given the stable and optimised nature of the patients recruited for this study, it was anticipated that baseline concentrations of all biomarkers were likely to be low, and potentially normal, for some of those enrolled. This being the case, relatively large increases in concentration would be required if categorical cut-off values were employed as the measurement of choice. With the small sample size, it was hypothesised that using this method could significantly limit the statistical analysis, as fewer events were deemed likely. By analysing absolute and percentage changes, results from all patients were guaranteed to be utilised for analytical comparison with the primary endpoint. Moreover, given that one of the main research objectives of this study was to examine biological variability in terms of RCV, assessment of percentage change would also provide meaningful comparison of such results.

3.6.3 Inferential Statistics

Wilcoxon signed ranks test was used to compare median biomarkers concentrations from baseline to each time point.

Pearson two-tailed statistic was used to identify significant correlations between biomarker levels and patient characteristics.

Receiver operating characteristic (ROC) analysis and area under curve (AUC) were used to evaluate the ability of a biomarker to identify those patients with a CV admission during the study period. Biomarker concentrations were considered a continuous variable for this analysis.

Cox proportional hazards analysis was used to identify the independent value of each biomarker as a predictor of CV admission.

Comparisons of groups by multi-marker scores were assessed using Kruskal-Wallis analysis.

SPSS v21 was used for all statistical analyses.

3.7 Biological variability

Several factors including pre-analytic, analytical variability and biological variability all contribute to total variability. In order to minimise pre-analytic factors, strict entry criteria (as described in section 3.1) were adopted in order to establish stability at baseline. In addition, patient preparation and blood collection protocol was standardised across the group. Blood samples were stored and assays performed in the minimum number of batches in order to reduce analytical variability.

3.7.1 Total Coefficient of Variation (CV_t)

The total coefficient of variation is composed of both analytic and biologic variation. Each patient's one-hour, one-month, three-month and six month CV_t was calculated from the standard deviation of the respective concentrations at baseline and one hour, baseline and one month, baseline and three months and baseline and six months.

As concentrations of all biomarkers were not normally distributed, data were log transformed prior to analysis.

3.7.2 Analytical Coefficient of Variation (CV_a)

Analytical coefficient of variation describes the reproducibility of the measurement of an analyte. Where possible, samples were analysed in a single series to minimise the contribution of inter-assay analytical variation. In accordance with laboratory quality control measures, assays were tested with repeated measures of control sera to determine the intra-assay coefficient of variation. These were provided by the laboratory, with mean intra-assay coefficient of variation being utilised as an estimate of overall CV_a .

3.7.3 Intra-individual Coefficient of Variation (CV_i)

Intra-individual coefficient of variation is the random variation that occurs around a homeostatic set point in an individual. CV_i was calculated according to the formula:

$$CV_i = (CV_t^2 - CV_a^2)^{1/2}.$$

3.7.4 Inter-individual Coefficient of Variation (CV_g)

Inter-individual coefficient of variation is the random variation that occurs between individuals and was calculated by dividing the standard deviation of the biomarker concentrations at each time point by the mean.

3.7.5 Reference Change Value (RCV)

Reference change values at a 95% confidence level were calculated from median CV_i values according to the formula:

$$RCV = Z \times 2^{1/2} (CV_i^2 + CV_a^2)^{1/2}$$

where Z (the 95% confidence interval Z score) is 1.96. This level of RCV reflects the minimum percentage change in serial results that is, with 95% confidence, different from the combined analytical and biological variation.

3.7.6 Index of Individuality (II)

Index of Individuality is the ratio of intra-individual to inter-individual variation and is calculated by the formula:

$$CV_i/CV_g$$

3.8 Ethics review and ethical conduct of the study

The study was performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice (GCP). The East Midlands National Research Ethics Service (NRES) Committee approved the study protocol. All patients were given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Patients provided signed and dated informed consent before any study specific procedure was conducted.

RESULTS

PATIENT CHARACTERISTICS

4.1 Baseline patient characteristics

Characteristics and mean medication doses of the 50 enrolled patients are shown in tables 8 and 9.

Clinical	
Age, mean (SD; Range)	67.26 (11.57; 45-87)
Male, n (%)	41 (82)
Ethnicity, n (%)	
Caucasian	41 (82)
Afro-Caribbean	7 (14)
Asian	2 (4)
Body mass index, mean (SD; Range)	29.98 (5.66; 20-45)
Hypertension, n (%)	25 (50)
Diabetes, n (%)	8 (16)
Ischaemic heart disease, n (%)	24 (48)
Atrial fibrillation, n (%)	12 (24)
QRS (ms), mean (SD; Range)	130.44 (38.38; 75-222)
CRT, n (%)	14 (28)
ICD, n (%)	18 (36)
NYHA I, n (%)	5 (10)
NYHA II, n (%)	35 (70)
NYHA III, n (%)	10 (20)
eGFR, mean (SD; Range)	64 (17.89; 26-91)
Na ⁺ , mean (SD; Range)	138 (3.01; 129-143)
Hb, mean (SD; Range)	13.7 (1.42; 10.9-17.4)
Medication	
ACE-I, n (%)	37 (74)
ARB, n (%)	13 (26)
β-blocker, n (%)	45 (90)
MRA, n (%)	34 (68)
Digoxin, n (%)	8 (16)
Regular Loop Diuretics, n (%)	37 (74)
Echo	
EF, mean (SD; Range)	30.68 (6.72; 14-40)
LVEDV, mean (SD; Range)	198.26 (64.34; 111-432)

Table 8: Baseline patient characteristics

Ischaemic heart disease (IHD) was defined as documented prior myocardial infarction, either with or without revascularisation, or prior history of either percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG).

Medication	Patients treated n (%)	Mean daily dose(mg)
ACE-I		
Ramipril	33 (66)	9.3
Enalapril	1 (2)	20
Lisinopril	1 (2)	10
Perindopril	2 (4)	4
ARB		
Candesartan	12 (24)	21.3
Valsartan	1 (2)	320
Beta blocker		
Bisoprolol	36 (72)	8.2
Carvedilol	7 (14)	58
Sotalol	2 (4)	120
MRA		
Spironolactone	19 (38)	27.6
Eplerenone	15 (30)	25.8

Table 9: Baseline mean prognostic medication doses

21 (42%) of patients had a QRS of ≥ 130 ms on baseline ECG. 14 (66.7%) patients with a QRS ≥ 150 ms were treated with CRT. One patient had been offered CRT but had declined at the time of recruitment. All the remaining 6 patients had QRS morphology of < 150 ms. Two demonstrated a right bundle branch block (RBBB) pattern on their ECG and had no device in situ, two demonstrated a non-specific interventricular conduction delay and were treated with an ICD, whilst a further two had conventional pacing devices but were either NYHA class I or had LVEF $> 35\%$.

Four patients withdrew from the study prior to completion of six-month follow-up. Reasons for withdrawal for consent included; one patient who was non-contactable after recruitment, two patients who decided the follow-up regime was not possible due to limitations in mobility and/or transport and one patient who left the country due to a family emergency abroad. Three patients who withdrew were followed up at the end of the study period. The medical records of the patient who remained uncontactable were obtained and the primary care physician was contacted to assess for evidence of any of the study endpoints. All available results were included in the final analysis. Blood samples from one patient at the one-hour review and one patient at the six-month review were misplaced after arrival in the lab and were therefore excluded from the final analysis.

8 (16%) of patients reached the primary end-point of cardiovascular admission, defined as admission due to decompensated HF, arrhythmia or acute coronary syndrome (table 10). No patient had more than one admission and no deaths occurred during the study period. Demographics of those patients with and without a CV admission are shown in table 11.

Patient	No. of admissions	Time from recruitment to admission (days)	Reason for admission
1	1	41	Fluid overload
2	1	147	Fluid overload
3	1	23	Fluid overload secondary to arrhythmia – PPM implanted
4	1	68	Fluid overload secondary to ACS – treated with PCI
5	1	26	Fluid overload secondary to ACS – treated with CABG/Mitral Valve Repair
6	1	13	Fluid overload secondary to ACS – medically managed
7	1	156	Fluid overload
8	1	44	Fluid overload secondary to arrhythmia – ICD implanted

Table 10: Admission details for the eight patients with a CV admission

	CV Admission	No CV Admission	p value
Clinical			
Age, mean (SD; Range)	68.13 (12.61; 50-87)	67.10 (11.52; 45-86)	0.820
Male, n (%)	4 (50)	37 (88)	0.011
Ethnicity, n (%)			
Caucasian	6 (75)	35 (83)	0.578
Afro-Caribbean	2 (25)	5 (12)	0.333
Asian	0 (0)	2 (5)	0.533
Body mass index, mean (SD; Range)	30.50 (5.66; 24-40)	29.88 (5.72; 20-45)	0.780
Hypertension, n (%)	3 (37.5)	22 (52.4)	0.445
Diabetes, n (%)	1 (12.5)	7 (16.7)	0.771
Ischaemic heart disease, n (%)	4 (50)	20 (47.6)	0.903
Atrial fibrillation, n (%)	2 (25)	10 (23.8)	0.943
QRS (ms), mean (SD; Range)	139 (44.64; 88-213)	129 (37.43; 75-222)	0.478
CRT, n (%)	2 (25)	12 (28.6)	0.838
ICD, n (%)	4 (50)	14 (33.3)	0.333
NYHA I, n (%)	0 (0)	5 (11.9)	0.309
NYHA II, n (%)	6 (75)	29 (69)	0.739
NYHA III, n (%)	2 (25)	8 (19)	0.703
eGFR, mean (SD; Range)	61 (20.10; 26-91)	65 (17.64; 31-91)	0.613
Na ⁺ , mean (SD; Range)	139 (1.69; 137-141)	138 (3.31; 129-143)	0.543
Hb, mean (SD; Range)	13.1 (1.44; 11.4-15.9)	13.8 (1.40; 10.9-17.4)	0.164
Medication			
ACE-I, n (%)	5 (62.5)	32 (76.2)	0.423
ARB, n (%)	3 (37.5)	10 (23.8)	0.423
β-blocker, n (%)	7 (87.5)	38 (90.5)	0.799
MRA, n (%)	7 (87.5)	27 (64.3)	0.202
Digoxin, n (%)	2 (25)	6 (14.3)	0.453
Regular Loop Diuretics, n (%)	8 (100)	29 (69)	0.070
Echo			
EF, mean (SD; Range)	27 (6.25; 19-36)	31 (6.68; 14-40)	0.116
LVEDV, mean (SD; Range)	243 (99.64; 138-432)	190 (52.87; 111-364)	0.032

Table 11: Baseline demographics of those with and without a CV admission

Patients who experienced a CV admission were more likely to be female ($p=0.011$), be on regular loop diuretics ($p=0.070$) and had significantly higher LVEDV ($p=0.032$) than those who did not.

MID-REGIONAL

PRO-ADRENOMEDULLIN

(MRproADM)

5.1 Introduction

The use of MRproADM for prognostication in HF patients has been well documented in a variety of settings^(222, 223, 225, 296-299). The use of serial measurements for monitoring, however, has been studied in only a few trials, with mixed results^(227, 300, 301). No studies have examined the biological variability of MRproADM in the chronic HF population or related this to the monitoring ability of MRproADM. This study attempts to address this gap in knowledge by calculating the biological variability of MRproADM and examining changes in MRproADM over a six-month period in a cohort of pharmacologically optimised patients with stable chronic HF.

5.2 Methods

This study was performed according to the methods and calculations set out in chapter 3 sections 3.1-3.2 and 3.5-3.8.

5.3 Results

5.3.1. Patient Characteristics

Characteristics of the 50 patients are described in section 4.1.

5.3.2 Biomarker concentrations

The distributions of all results for NTproBNP and MRproADM were non-parametric. Median concentrations of NTproBNP and MRproADM at each visit are detailed in table 12. There were no significant differences in median values from baseline across any time point for NTproBNP (one hour $p=0.874$, one month $p=0.883$, three months $p=0.144$, six months $p=0.279$). Compared with baseline, no significant difference was found for median MRproADM concentrations at one hour ($p=0.074$), one month ($p=0.105$) or three months ($p=0.084$). A significant difference was, however, demonstrated at six months ($p=0.001$).

	Baseline	One Hour	One Month	Three Months	Six Months
NTproBNP	300	285	466	291	356
(ng/L)	(80.8-1282)	(90.6-1150)	(80.2-1171)	(46.5-1006)	(61.9-1469)
MRproADM	0.73	0.67	0.74	0.75	0.79
(nmol/L)	(0.58-0.95)	(0.49-1.02)	(0.60-1.03)	(0.62-1.03)	(0.63-1.22)

Table 12: Median concentrations of NTproBNP and MRproADM at each time point

BIOLOGICAL VARIABILITY

5.4.1 Analytical Coefficient of Variation (CV_a)

Mean intra-assay coefficient of variation was utilised as an estimate of overall CV_a . Using this calculation, the CV_a 's for NTproBNP and MRproADM were 4.17% and 4.88% respectively.

5.4.2 Individual Coefficients of Variation, Index of Individuality and Reference Change Values

CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and MRproADM at each time point are shown in table 13.

	1 Hour		1 Month		3 Months		6 Months	
	NTproBNP	MRproADM	NTproBNP	MRproADM	NTproBNP	MRproADM	NTproBNP	MRproADM
CV_i	18.47	14.09	36.75	13.11	40.98	14.04	46.02	18.93
CV_g	28	48	28	42	32	38	32	63
II	0.65	0.29	1.31	0.31	1.28	0.37	1.44	0.30
RCV (%)	52	41	103	39	114	41	128	54

Table 13: CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and MRproADM at each time point

Paired t-tests were used to examine differences in CV_i across the time points. Compared with one hour CV_i , significant variability was seen across all time points for NTproBNP; one hour to one month $p=0.003$, one hour to three months $p<0.001$, and one hour to six months $p=0.003$. No significant variability was demonstrated between any time points for MRproADM.

No significant difference was demonstrated between CV_i for NTproBNP and MRproADM at one hour ($p=0.610$). Significant differences were, however, observed between CV_i for NTproBNP and MRproADM at all other points; one month $p=0.002$, three months $p<0.001$ and six months $p<0.001$.

5.4.3 Individual Coefficients of Variation, Index of Individuality and Reference Change Values:

Stable Patients

CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and MRproADM at each time point were re-calculated after removal of any patients reaching the primary end-point of the study. Results are shown in table 14.

	1 Hour		1 Month		3 Months		6 Months	
	NTproBNP	MRproADM	NTproBNP	MRproADM	NTproBNP	MRproADM	NTproBNP	MRproADM
CV_i	18.47	14.09	36.15	12.65	41.54	12.41	42.92	11.93
CV_g	28	48	29	37	32	38	34	30
II	0.65	0.29	1.25	0.34	1.28	0.33	1.39	0.39
RCV (%)	52	41	100	35	115	34	120	33

Table 14: CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and MRproADM at each time point for patients not experiencing a CV admission.

Paired t-tests were used to examine differences in CV_i across the time points. After removal of patients experiencing a CV admission, variability for NTproBNP remained significant across all time points; one hour to one month $p=0.029$, one hour to three months $p=0.019$ and one hour to six months $p=0.022$. No significant variability was demonstrated for MRproADM between one hour and one month ($p=0.868$) or one hour and three months ($p=0.933$), however, variability for MRproADM was now demonstrated between one hour and six months ($p=0.014$).

No significant difference was demonstrated between CV_i for NTproBNP and MRproADM at one hour ($p=0.610$). As with results prior to removal of patients experiencing a CV admission, significant differences were observed between CV_i for NTproBNP and MRproADM at all other time points; one month $p=0.002$, three months $p=0.001$ and six months $p=0.005$.

SERIAL MONITORING

5.5.1 Baseline correlations

Pearson correlation was carried out to evaluate the relationship of baseline NTproBNP and MRproADM with several patient characteristics related to prognosis (table 15).

In keeping with previous studies on NTproBNP, baseline concentrations significantly correlated with LVEF ($p=0.001$), end diastolic volume (EDV) ($p=0.011$), duration of QRS complex on 12 lead ECG ($p=0.008$), NYHA class ($p=0.042$), creatinine ($p=0.006$) and eGFR ($p=0.012$). MRproADM significantly correlated with NTproBNP ($p<0.001$), age ($p<0.001$), duration of QRS complex on 12 lead ECG ($p=0.003$), creatinine ($p=0.005$) and eGFR ($p=0.001$).

	NTproBNP	MRproADM
NTproBNP	1.00	0.486**
MRproADM	0.486**	1.00
Age	0.251	0.504**
LVEF	-0.439**	-0.194
LVEDV	0.355*	0.146
NYHA Class	0.289*	0.272
HR	-0.018	-0.043
QRS	0.372**	0.415**
Creatinine	0.382**	0.390**
eGFR	-0.352*	-0.459**

Table 15: Pearson correlation of baseline NTproBNP and MRproADM with patient characteristics related to prognosis (*significant at the 0.05 level, ** significant at the 0.01 level)

5.5.2 Single absolute concentrations and cardiovascular events

ROC analysis of single absolute concentrations at baseline, one month and three months showed

NTproBNP to be better than MRproADM at predicting CV admission over the six month study period (figures 19 and 20) (AUC 0.764; 95% CI 0.619 to 0.910; $p=0.028$, AUC 0.780; 95% CI 0.636 to 0.924; $p=0.020$ and AUC 0.714; 95% CI 0.540 to 0.889; $p=0.075$ vs. AUC 0.660; 95% CI 0.417 to 0.902; $p=0.160$, AUC 0.525; 95% CI 0.248 to 0.801; $p=0.828$ and AUC 0.638; 95% CI 0.399 to 0.878; $p=0.224$ respectively).

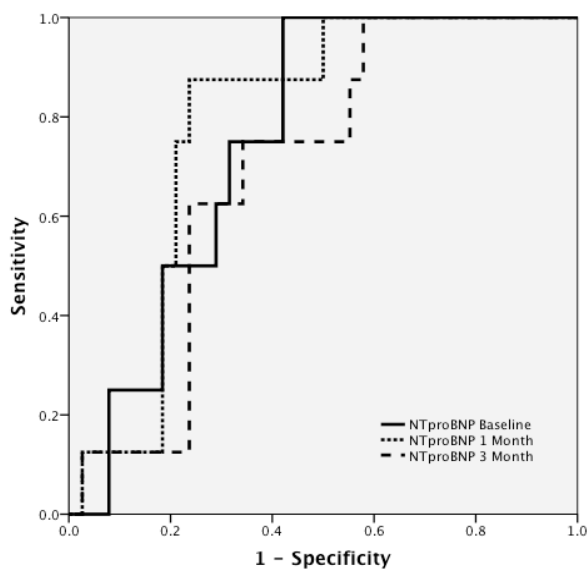


Figure 19: ROC analysis of single absolute NTproBNP concentrations and CV admission

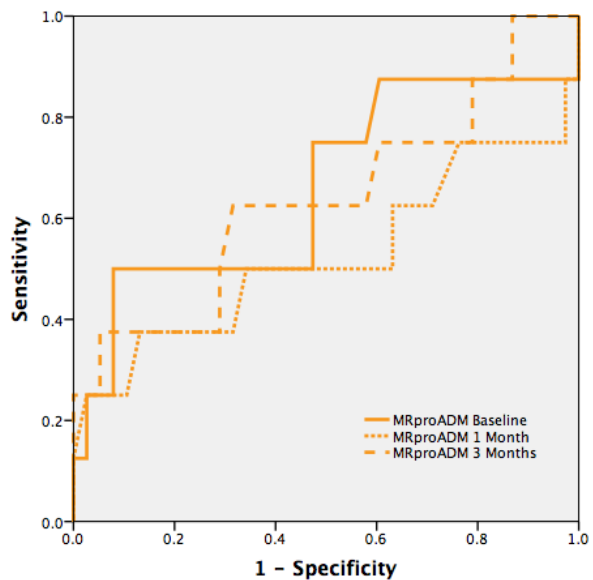


Figure 20: ROC analysis of single absolute MRproADM concentrations and CV admission

5.5.3 Performance characteristics of serial MRproADM as a discriminator of patient risk

The value of relative changes in both NTproBNP and MRproADM were assessed by considering absolute and percentage changes from baseline to follow-up measurement. Median (Interquartile Range (IQR)) percentage changes for the group overall and for those with and without a CV admission are shown in table 16. A lower MRproADM was observed in those experiencing a CV admission at one month and three months, however no statistical significance was demonstrated. Boxplot analyses (figures 21 and 22) of percentage change in MRproADM and NTproBNP over the six-month study period demonstrates the variation in biomarker concentrations for those experiencing a CV admission, compared with those who did not.

Time		Overall n	Overall Median % change	CV Adm (n)	CV Adm Median % change (IQR)	No CV Adm (n)	No CV Adm Median % change	p value
Baseline		50	-	-	-	-	-	-
			-		-		-	
1 month	MRproADM	47	3.2 (-7.8-19.8)	3	-9.6 (-12.2-0.0)	44	4.7 (-3.1-21.8)	0.415
	NTproBNP		-7.2 (-28.6-44.0)		13.2 (-54.4-82.9)		-8.9 (-28.5-50.4)	0.822
3 months	MRproADM	46	6.6 (-8.3-24.5)	6	-11.4 (-19.9-59.5)	40	8.2 (-7.5-23.7)	0.313
	NTproBNP		-21.4 (-49.2-38.3)		-41.5 (-65.9 - -7.3)		-9.1 (-45.1-44.3)	0.811
6 months	MRproADM	45	11.0 (-4.1-48.5)	7	6.8 (-4.2-141.4)	38	11.8 (-4.0-47)	0.975
	NTproBNP		8.9 (-22.7-66.2)		39.8 (-24.6-138.9)		5.1 (-28.6-59.5)	0.571

Table 16: Comparison of median interquartile range (IQR) percentage changes for NTproBNP and MRproADM overall and for those with and without a CV admission

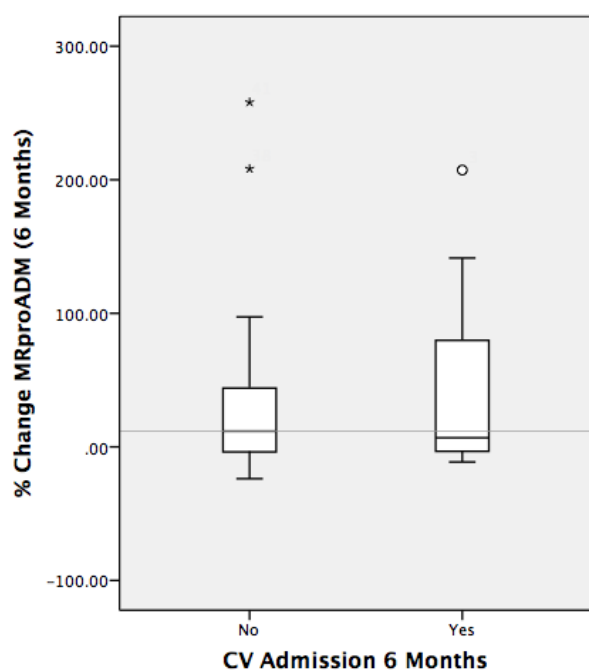


Figure 21: Box-plot analysis of % change in MRproADM over six months according to CV admission status (o=outlier 1.5 x IQR, ★= outlier 3 x IQR)

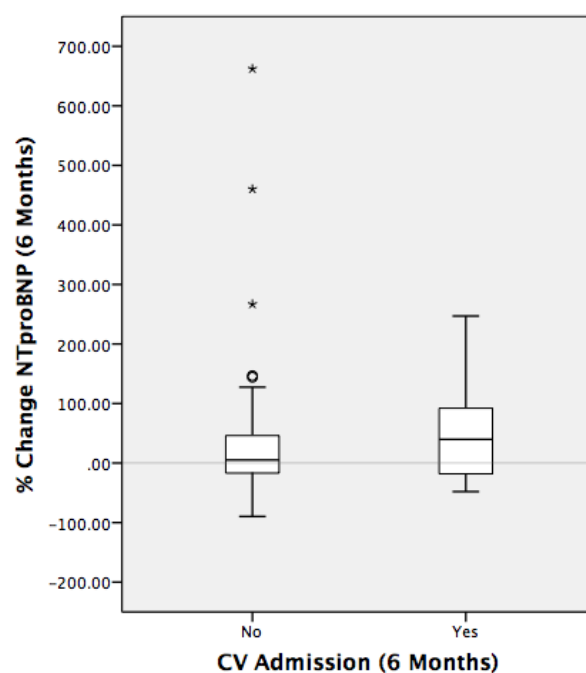


Figure 22: Box-plot analysis of % change in NTproBNP over six months according to CV admission status (o=outlier 1.5 x IQR, ★= outlier 3 x IQR)

ROC analysis of absolute changes showed MRproADM was no better than NTproBNP at predicting CV admission across the time points - one month (AUC 0.360; 95% CI 0.021 to 0.699; $p=0.421$ vs. AUC 0.538; 95% CI 0.133 to 0.943; $p=0.828$) (figure 23), three months (AUC 0.369; 95% CI 0.052 to 0.686; $p=0.304$ vs. AUC 0.296; 95% CI 0.046 to 0.546; $p=0.110$) (figure 24), and six months (AUC 0.546; 95% CI 0.298 to 0.795; $p=0.700$ vs. AUC 0.579; 0.282 to 0.877; $p=0.511$) (figure 25).

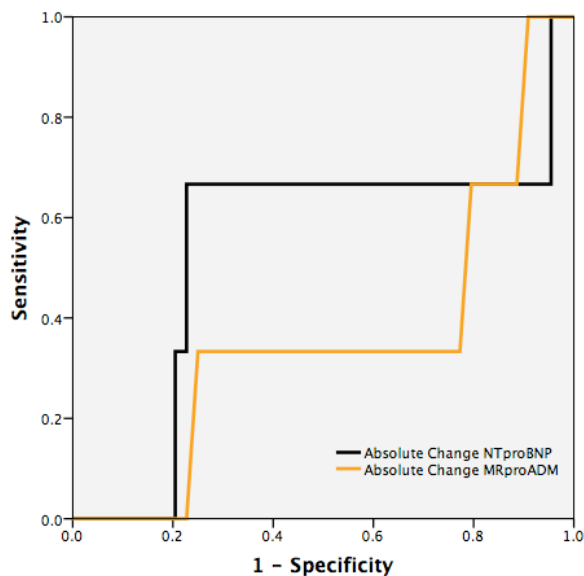


Figure 23: ROC analysis of absolute change in MRproADM and NTproBNP over one month and CV admission

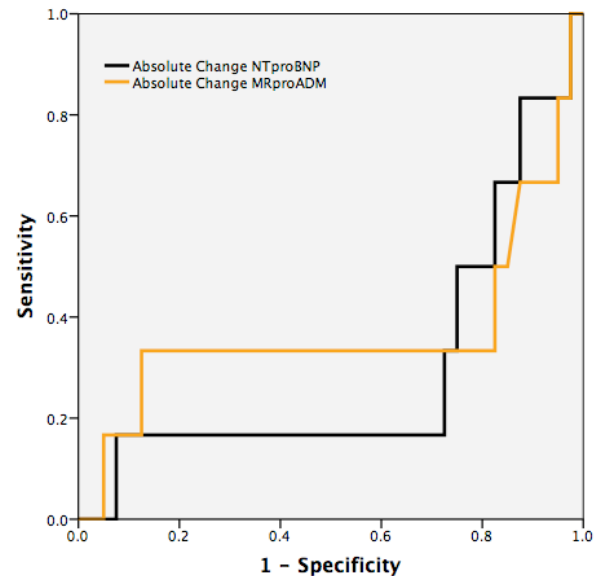


Figure 24: ROC analysis of absolute change in MRproADM and NTproBNP over three months and CV admission

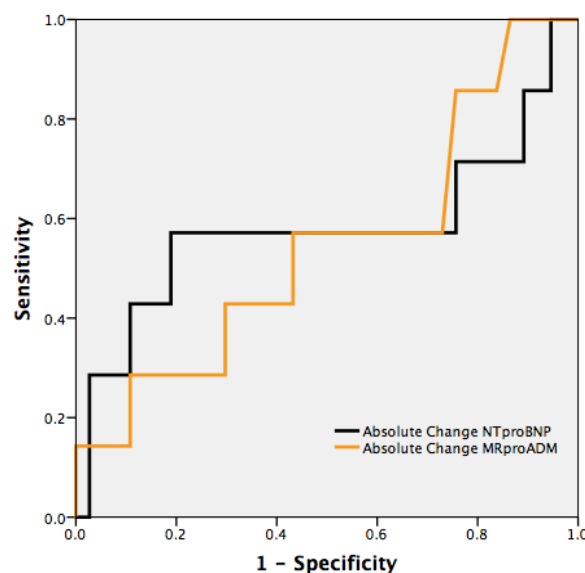


Figure 25: ROC analysis of absolute change in MRproADM and NTproBNP over six months and CV admission

Similar results were demonstrated for percentage change in MRproADM. At all time points MRproADM was less predictive of CV admission than NTproBNP - one month (AUC 0.348; 95% CI 0.029 to 0.668; $p=0.384$ vs. AUC 0.455; 95% CI 0.142 to 0.767; $p=0.159$) (figure 26), three months (AUC 0.367; 95% CI 0.037 to 0.696; $p=0.297$ vs. AUC 0.325; 95% CI 0.100 to 0.550; $p=0.171$) (figure 27) and six months (AUC 0.506; 95% CI 0.254 to 0.757; $p=0.962$ vs. AUC 0.571; 95% CI 0.341 to 0.802; $p=0.553$) (figure 28).

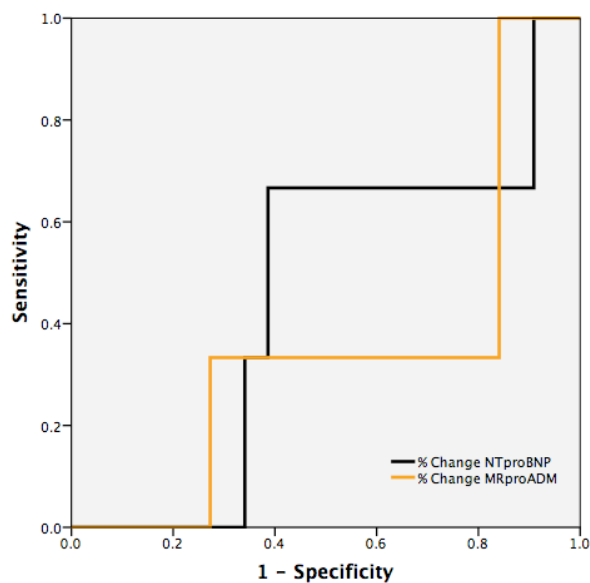


Figure 26: ROC analysis of % change in MRproADM and NTproBNP over one month and CV admission

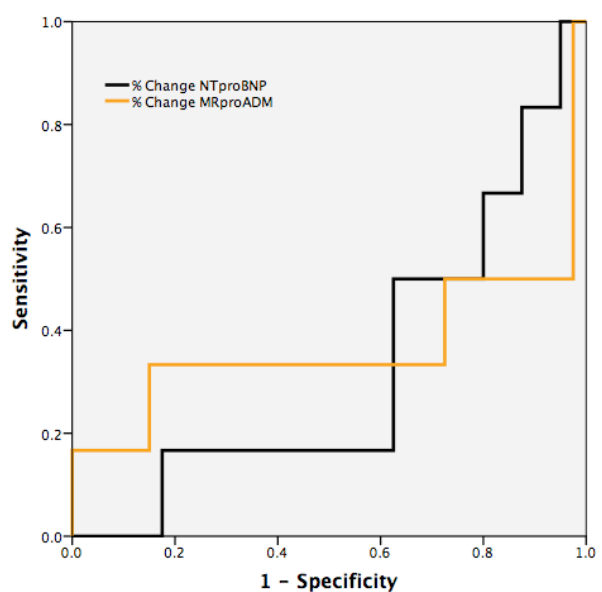


Figure 27: ROC analysis of % change in MRproADM and NTproBNP over three months and CV admission

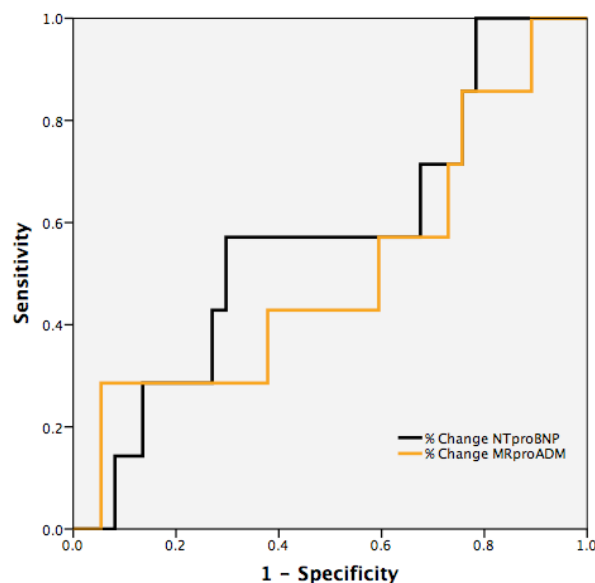


Figure 28: ROC analysis of % change in MRproADM and NTproBNP over six months and CV admission

5.5.4 MRproADM concentrations and renal function

At each time point, single MRproADM concentrations were significantly correlated with both creatinine (baseline $p=0.005$, one month $p=0.003$, three months $p<0.001$, six months $p<0.001$) and eGFR (baseline $p=0.001$, one month $p<0.001$, three months $p<0.001$, six months $p<0.001$).

On ROC analysis, no significant relationship between absolute changes in either MRproADM or NTproBNP and worsening renal function (increase in creatinine $\geq 25\%$) was demonstrated at either three months (AUC 0.694; 95% CI 0.497 to 0.890; $p=0.266$ vs. AUC 0.736; 95% CI 0.480 to 0.993; $p=0.175$) or six months (AUC 0.787; 95% CI 0.574 to 0.999; $p=0.061$ and AUC 0.573; 95% CI 0.233 to 0.913; $p=0.632$). Insufficient events occurred at one month to allow meaningful analysis.

Similar results were seen for percentage change in MRproADM and NTproBNP at three months with AUC 0.659; 95% CI 0.506 to 0.812; $p=0.362$ vs. AUC 0.651; 95% CI 0.217 to 1.00; $p=0.386$. Percentage change in MRproADM and worsening renal function at six months, however, showed an apparent difference compared to NTproBNP and almost reached significance, with an AUC of 0.794; 95% CI 0.615 to 0.973; $p=0.05$) (figure 29).

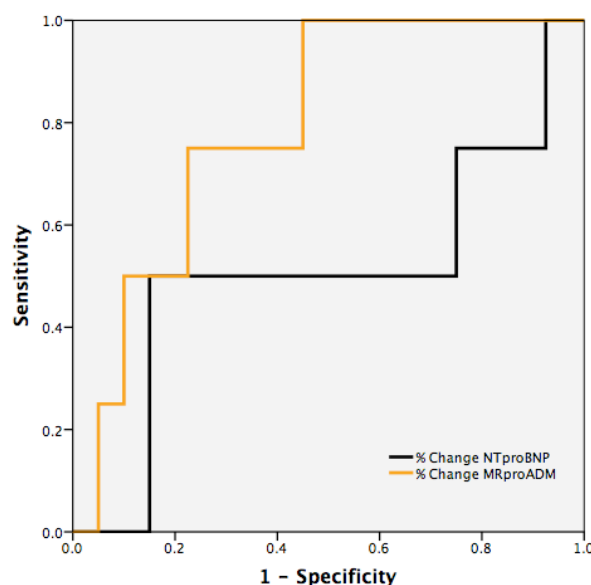


Figure 29: ROC analysis of % change in MRproADM and NTproBNP over six months and worsening renal function

5.6 Discussion

In contrast to NTproBNP, MRproADM does not exhibit significant variation either in the short or long term with RCVs ranging from 41% to 54%, compared with 52% to 128% for NTproBNP. However, after removal of all patients who experienced a CV admission, results did demonstrate significant differences in the calculated RCVs for MRproADM between one hour and six months. Clearly whilst this could have implications for serial monitoring, it is unlikely that any monitoring policy would include sampling at time points as short as one hour. Although not assessed in this study, it would be necessary to evaluate BV and RCV at a minimum of two weeks, the current recommended follow-up time post discharge following acute decompensation in the UK⁽³⁰²⁾, in order to establish reliable BV components. Given that serial monitoring is unlikely to take place at time points of one hour, this finding is unlikely to detract from the potential for monitoring that these results imply. Further support for this can be taken from the low index of individuality of MRproADM, indicating significant differences between individuals relative to the population. Consequently, single concentrations cannot be effectively compared to a population-based reference range - with serial sampling relative to the individual providing a more meaningful comparison. From a monitoring perspective, these findings indicate that MRproADM may be better for this purpose than NTproBNP.

Results from the monitoring aspect of the study, however, did not prove this to be the case. Despite significantly lower biological variability and references changes values than NTproBNP, and significant differences between median concentration of MRproADM between baseline and six-months, neither absolute nor percentage changes over the same time frame were predictive for CV admission, with ROC analysis of such changes showing MRproADM to be worse or equal to that of NTproBNP.

These results corroborate those of Neuhold et al, who demonstrated that changes in MRproADM

measured before and after medical optimisation had no predictive value for all-cause mortality⁽²²⁷⁾. More recently, reports from the VERDY trial showed that, in 441 patients presenting with dyspnoea, changes in MRproADM over 72hrs during hospitalisation were non-significant for predicting events at 30 and 90-day follow-up⁽³⁰³⁾. However, of these, only 27% were known chronic HF. Indeed, only one published study has demonstrated any ability of changes in MRproADM to predict HF events. In a study of over 6,000 patients with stable CAD, the LIPID trial demonstrated that changes in MRproADM concentrations over one year were associated with risk of HF, with net reclassification improvement of 5.60%⁽³⁰⁴⁾. Given the large patient cohort, such findings cannot be ignored and may well be more representative of the predictive ability of MRproADM than both my own and previous, much smaller, studies. It should be noted, however, that this trial made no attempt to classify LVSD and, more importantly, actively excluded any patients with known EF < 25%.

One potential signal towards a positive result is seen with changes in MRproADM and renal function. Over the six-month study period, percentage change in MRproADM was shown to be associated with deterioration in renal function, defined as a $\geq 25\%$ increase in creatinine. Although this result failed to reach statistical significance, previous studies of MRproADM have indicated a likely relationship with renal function, which would be consistent with this result^(300, 301, 305-308). Moreover, results from the study presented here demonstrate that concentrations of MRproADM at each time point were strongly positively correlated with both creatinine and eGFR. Although neither changes in creatinine or eGFR were predictive of CV admission, renal dysfunction per se is a well-recognised prognostic marker in HF⁽³⁰⁹⁻³¹⁵⁾ and the ability to detect sub-clinical changes which do not result in admission but are prognostic with respect to long term outcomes may have significant benefits in terms of monitoring patients who are otherwise fully optimised. Further, larger trials examining this effect and its association with outcomes are required to validate this hypothesis.

5.7 Conclusion

Despite significantly lower biological variability than NTproBNP, percentage and absolute changes in MRproADM are poor predictors of CV admission in patients with pharmacologically optimised stable chronic HF. Results of this study reveal it may be worse than that of NTproBNP, and as such an unattractive marker for future use as a monitoring tool for this purpose. MRproADM may, however, have uses for detecting changes in renal function prior to such changes resulting in decompensated HF requiring hospital admission. Larger trials are required in order to validate these findings.

Apelin

6.1 Introduction

Studies examining the use of apelin as a biomarker of HF have produced mixed results. Whilst several studies have demonstrated reduced plasma and myocardial apelin levels in patients with advanced HF^(245, 247, 248, 290, 316-318), plasma apelin concentrations do not appear to be predictive of outcome in such patients⁽³¹⁹⁾. Moreover, other studies have failed to show any significant difference in plasma apelin concentrations between patients with stable dilated cardiomyopathy and normal controls⁽²⁴⁴⁾ or in those presenting with acute HF⁽²⁴³⁾. No studies have attempted to assess the biological variability of apelin, or assess serial measurements for monitoring potential.

This study is the first to examine the calculated biological variability with the ability of serial changes to predict CV admission over a six-month follow-up period in the same cohort of pharmacologically optimised stable HF patients (NYHA I-III).

6.2 Methods

This study was performed according to the methods and calculations set out in chapter 3 sections 3.1-3.2 and 3.5-3.8.

6.3 Results

6.3.1 Patient Characteristics

Characteristics of the 50 patients are described in section 4.1.

6.3.2 Biomarker Concentrations

The distributions of all results for NTproBNP and apelin were non-parametric. Median concentrations of NTproBNP and apelin at each visit are detailed in table 17. There were no significant differences in median concentrations from baseline across the time points for either

NTproBNP (one hour p=0.874, one month p=0.883, three months p=0.144, six months p=0.279) or apelin (one hour p=0.988, one month p=0.169, three months p=0.074, six months p=0.056).

	Baseline	One Hour	One Month	Three Months	Six Months
NTproBNP	300	285	466	291	356
(ng/L)	(80.8-1282)	(90.6-1150)	(80.2-1171)	(46.5-1006)	(61.9-1469)
Apelin 12	201	183	216	217	242
(ng/L)	(99.8-354)	(123-355)	(122-410)	(141-503)	(119-448)

Table 17: Median concentrations of NTproBNP and apelin at each time point

BIOLOGICAL VARIABILITY

6.4.1 Analytical Coefficient of Variation (CV_a)

Mean intra-assay coefficient of variation was utilised as an estimate of overall CV_a . Using this calculation, the CV_a 's for NTproBNP and Apelin were 4.17% and 3.53% respectively.

6.4.2 Individual Coefficients of Variation, Index of Individuality and Reference Change Values

CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and apelin at each time point are shown in table 18.

	1 Hour		1 Month		3 Months		6 Months	
	NTproBNP	Apelin	NTproBNP	Apelin	NTproBNP	Apelin	NTproBNP	Apelin
CV_i	18.47	6.84	36.75	9.23	40.98	9.49	46.02	10.19
CV_g	28	14	28	16	32	15	32	16
II	0.65	0.48	1.31	0.58	1.28	0.63	1.44	0.64
RCV (%)	52	21	103	27	114	28	128	30

Table 18: CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and apelin at each time point

Paired t-tests were used to examine differences in CV_i across the time points. Compared with one hour CV_i , significant variability was seen across all time points for NTproBNP; one hour to one month $p=0.003$, one hour to three months $p<0.001$, and one hour to six months $p=0.003$. Apelin CV_i results showed significant differences only between one hour and six months; one hour to one month $p=0.057$, one hour to three months $p=0.077$ and one hour to six months $p=0.007$.

Significant differences were observed between CV_i for NTproBNP and apelin at all time points; one hour $p=0.036$, one month $p<0.001$, three months $p<0.001$ and six months $p<0.001$.

6.4.3 Individual Coefficients of Variation, Index of Individuality and Reference Change Values:

Stable Patients

CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and apelin at each time point were re-calculated after removal of any patients reaching the primary end-point of the study. Results are shown in table 19.

	1 Hour		1 Month		3 Months		6 Months	
	NTproBNP	Apelin	NTproBNP	Apelin	NTproBNP	Apelin	NTproBNP	Apelin
CV_i	18.47	6.84	36.15	9.58	41.54	10.55	42.92	10.19
CV_g	28	14	29	16	32	14	34	16
II	0.65	0.48	1.25	0.61	1.28	0.74	1.39	0.66
RCV								
(%)	52	21	100	27	115	29	120	28

Table 19: CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and apelin at each time point for patients not experiencing a CV admission.

Paired t-tests were used to examine differences in CV_i across the time points. After removal of patients experiencing a CV admission, variability for NTproBNP remained significant across all time points; one hour to one month $p=0.029$, one hour to three months $p=0.019$ and one hour to six months $p=0.022$. Apelin CV_i results, however, subsequently also showed significant differences across all time points; one hour to one month $p=0.003$, one hour to three months $p=0.018$ and one hour to six months $p=0.007$.

Significant differences were observed between CV_i for NTproBNP and apelin at all time points; one hour $p=0.036$, one month $p<0.001$, three months $p<0.001$ and six months $p<0.006$.

SERIAL MONITORING

6.5.1 Baseline correlations

Pearson correlation was carried out to evaluate the relationship of baseline NTproBNP and apelin with several patient characteristics related to prognosis (table 20). In keeping with previous studies on NTproBNP, baseline concentrations significantly correlated with LVEF ($p=0.001$), end diastolic volume (EDV) ($p=0.011$), duration of QRS complex on 12 lead ECG ($p=0.008$), NYHA class ($p=0.042$), creatinine ($p=0.006$) and eGFR ($p=0.012$).

Apelin was found only to correlate significantly with creatinine ($p=0.048$).

	NTproBNP	Apelin
NTproBNP	1.00	0.244
Apelin	0.244	1.00
Age	0.251	0.176
LVEF	-0.439**	0.003
LVEDV	0.355*	-0.157
NYHA Class	0.289*	0.267
HR	-0.018	-0.142
QRS	0.372**	0.130
Creatinine	0.382**	0.281*
eGFR	-0.352*	-0.245

Table 20: Pearson correlation of baseline NTproBNP and apelin with patient characteristics related to prognosis (* significant at the 0.05 level, ** significant at the 0.01 level)

6.5.2 Single absolute concentrations and cardiovascular events

ROC analysis of single absolute concentrations at baseline, one month, and three months showed NTproBNP to be better than apelin at predicting CV admission over the six month study period

(figures 30 and 31) (AUC 0.764; 95% CI 0.619 to 0.910; $p=0.028$, AUC 0.780; 95% CI 0.636 to 0.924; $p=0.020$ and AUC 0.714; 95% CI 0.540 to 0.889; $p=0.075$ vs. AUC 0.641; 95% CI 0.470 to 0.813; $p=0.213$, AUC 0.470; 95% CI 0.277 to 0.664; $p=0.794$ and AUC 0.538; 95% CI 0.322 to 0.753; $p=0.739$ respectively).

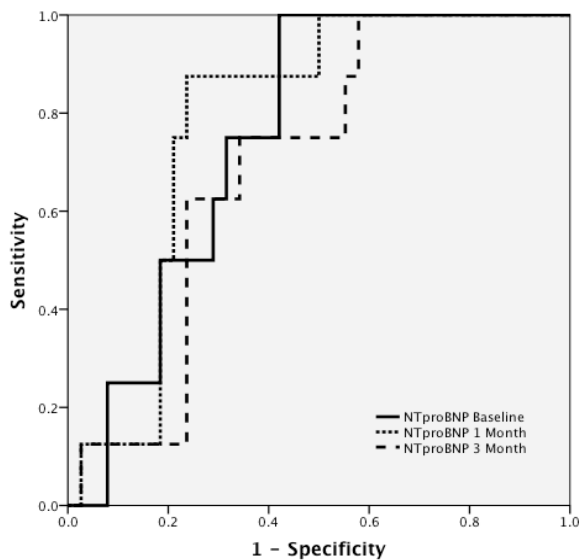


Figure 30: ROC analysis of single absolute NTproBNP concentrations and CV admission

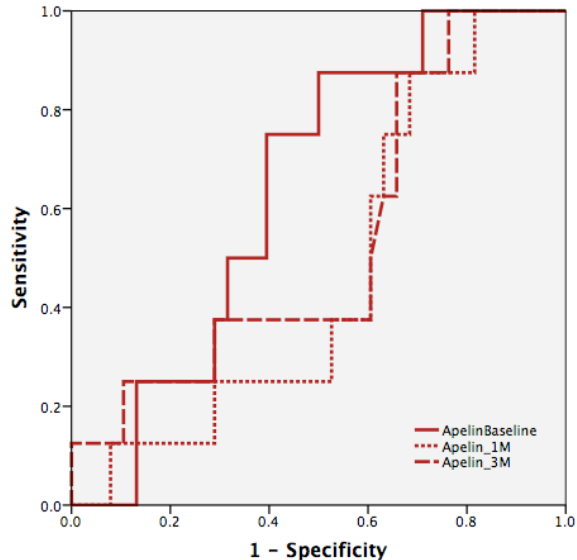


Figure 31: ROC analysis of single absolute apelin concentrations and CV admission

6.5.3 Performance characteristics of serial apelin as a discriminator of patient risk

The value of relative changes in both NTproBNP and apelin were assessed by considering absolute and percentage changes from baseline to follow-up measurement. Median (Interquartile Range (IQR)) percentage changes for the group overall and for those with and without a CV admission are shown in table 21. Although differences were observed at each time point for both apelin and NTproBNP between those experiencing a CV admission and those who did not, no statistical significance was demonstrated.

Boxplot analyses (figures 32 and 33) of percentage change in apelin and NTproBNP over the six-month study period demonstrate the variation in biomarker concentrations for those experiencing a CV admission, compared with those who did not.

Time		Overall n	Overall Median % change	CV Adm (n)	CV Adm Median % change (IQR)	No CV Adm (n)	No CV Adm Median % change	p value
Baseline		50	-	-	-	-	-	-
			-		-		-	
1 month	Apelin	47	11.1 (-34.6-51.5)	3	-31.5 (-34.6-0.0)	44	13.1 (-47.5-50.4)	0.919
	NTproBNP		-7.2 (-28.6-44.0)		13.2 (-54.4-82.9)		-8.9 (-28.5-50.4)	0.822
3 months	Apelin	46	9.5 (42.3-58.2)	6	27.3 (-66.1-57.0)	40	3.9 (-42.7-58.3)	0.987
	NTproBNP		-21.4 (-49.2-38.3)		-41.5 (-65.9 - -7.3)		-9.1 (-45.1-44.3)	0.811
6 months	Apelin	45	19.4 (-63.2-57.5)	7	35.2 (-30.2-60.5)	38	18.3 (-84.5-57.0)	0.570
	NTproBNP		8.9 (-22.7-66.2)		39.8 (-24.6-138.9)		5.1 (-28.6-59.5)	0.571

Table 21: Comparison of median interquartile range (IQR) percentage changes for NTproBNP and apelin overall and for those with and without a CV admission

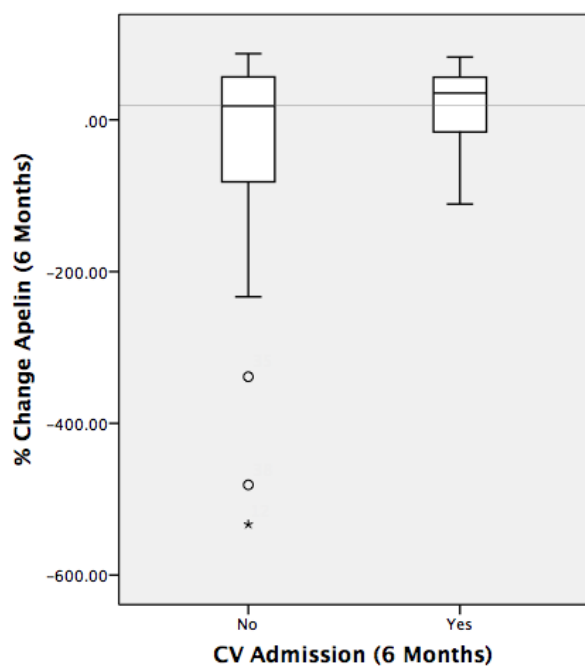


Figure 32: Box-plot analysis of % change in apelin over 6 months according to CV admission status (o=outlier 1.5 x IQR, ★= outlier 3 x IQR)

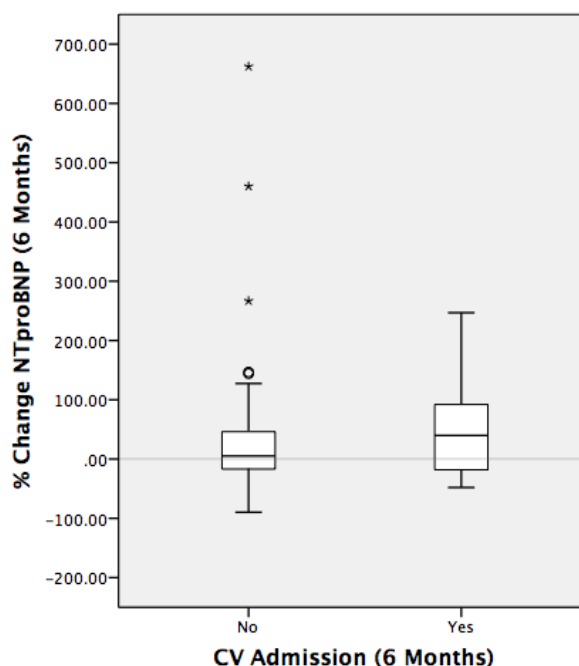


Figure 33: Box-plot analysis of % change in NTproBNP over 6 months according to CV admission status (o=outlier 1.5 x IQR, ★= outlier 3 x IQR)

ROC analysis of absolute changes showed apelin was no better than NTproBNP at predicting CV admission at one month (AUC 0.508; 95% CI 0.172 to 0.843; $p=0.965$ vs. AUC 0.538; 95% CI 0.133 to 0.943; $p=0.828$) (figure 34), three months (AUC 0.483; 95% CI 0.273 to 0.694; $p=0.896$ vs. AUC 0.296; 95% CI 0.046 to 0.546; $p=0.110$) (figure 35), or six months (AUC 0.417; 95% CI 0.188 to 0.646; $p=0.490$ vs. AUC 0.579; 95% CI 0.282 to 0.877; $p=0.511$) (figure 36).

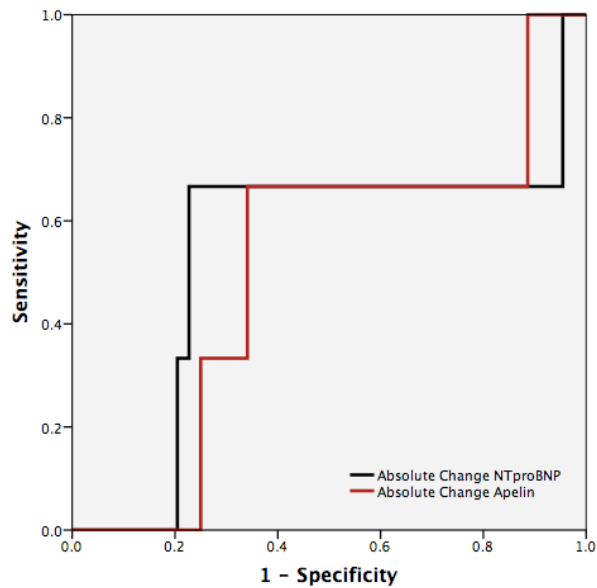


Figure 34: ROC analysis of absolute changes in apelin and NTproBNP over one month and CV admission

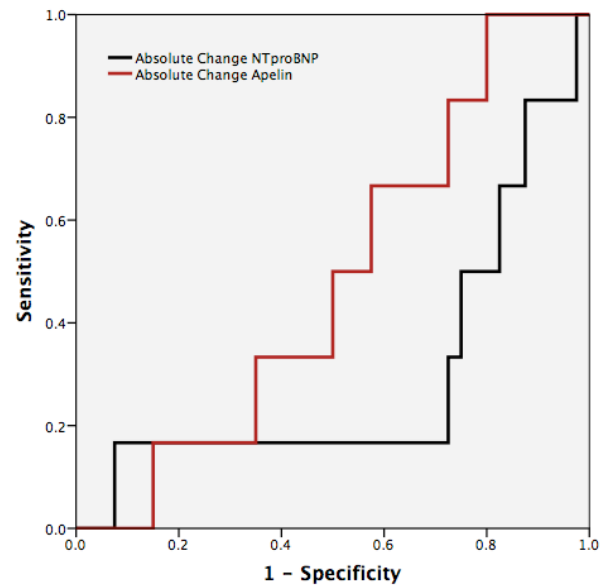


Figure 35: ROC analysis of absolute changes in apelin and NTproBNP over three months and CV admission

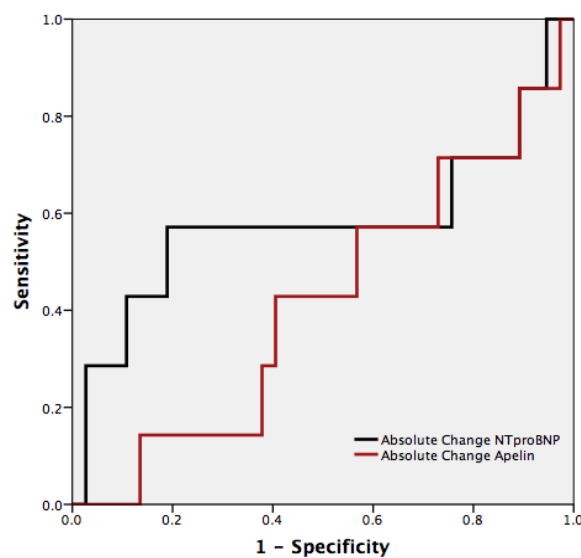


Figure 36: ROC analysis of absolute changes in apelin and NTproBNP over six months and CV admission

Similar results were demonstrated for percentage change in apelin, with no significant ability to predict CV admission at any of the examined time points – one month AUC 0.477; 95% CI 0.117 to 0.838; $p=0.896$ vs. AUC 0.455; 95% CI 0.142 to 0.767; $p=0.794$ (figure 37), three month AUC 0.504; 95% CI 0.275 to 0.734; $p=0.974$ vs. AUC 0.325; 95% CI 0.100 to 0.550; $p=0.171$ (figure 38) and six months, where apelin was equal to that of NTproBNP at predicting CV admission with AUC of 0.571, $p=0.553$ for both biomarkers (95% CI 0.352 to 0.790 and 0.341 to 0.802 respectively) (figure 39).

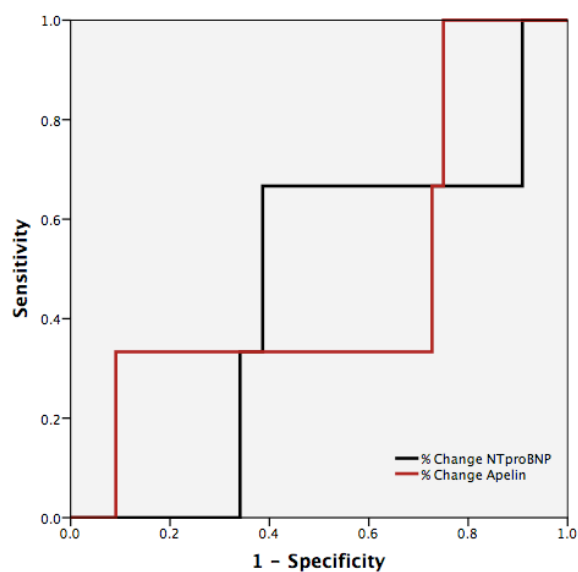


Figure 37: ROC analysis of % change in apelin and NTproBNP over one month and CV admission

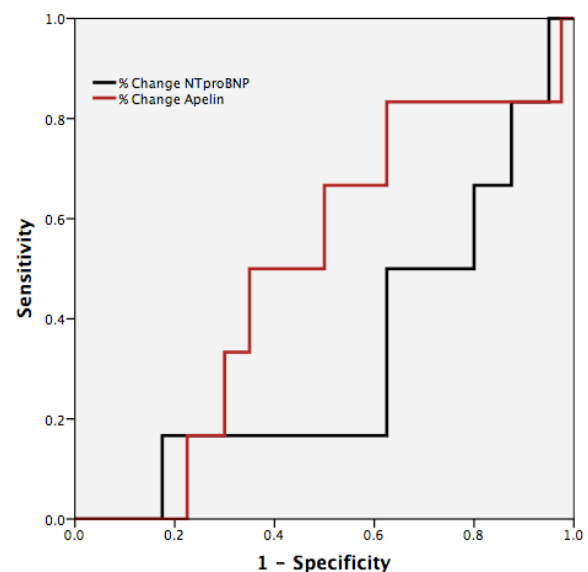


Figure 38: ROC analysis of % changes in apelin and NTproBNP over three months and CV admission

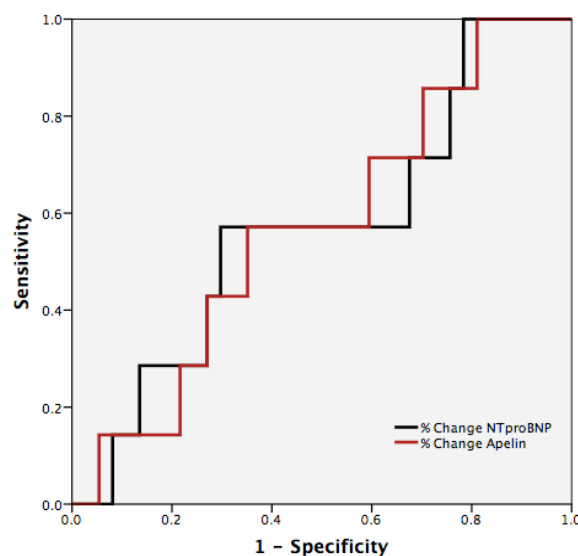


Figure 39: ROC analysis of % change in apelin and NTproBNP over six months and CV admission

6.5.4 Apelin concentrations and renal function

Despite the correlation observed between baseline apelin and creatinine, no other correlation was seen at any other time point.

Insufficient events occurred at one month to allow meaningful analysis at this time point. ROC analysis showed no significant relationship between absolute or percentage change concentrations of apelin and worsening renal function ($\geq 25\%$ increase in creatinine) at either three months (AUC 0.791; 95% CI 0.639 to 0.942; $p=0.095$ and AUC 0.209; 95% CI 0.070 to 0.349; $p=0.095$) or six months (AUC 0.476; 95% CI 0.233 to 0.718; $p=0.873$ and AUC 0.543; 95% CI 0.259 to 0.826; $p=0.780$).

6.6 Discussion

Compared with NTproBNP, apelin demonstrates significantly lower biological variability. However, results also demonstrate significant differences in the calculated RCV for apelin between one hour and six months. Clearly whilst this could have implications for serial monitoring, it is unlikely that any monitoring policy would include sampling at time points as short as one hour. Although not assessed in this study, it would be necessary to evaluate BV and RCV at a minimum of two weeks, the current recommended follow-up time post discharge following acute decompensation in the UK⁽³⁰²⁾, in order to establish reliable BV components. Following removal of all patients who experienced a CV admission, significant differences were, however, demonstrated across all time points, indicating that difference RCVs would be required according to the time between samples if apelin were to be employed for serial monitoring. Although not an insurmountable disadvantage, from a practical perspective such results indicate that apelin may be no better than NTproBNP for monitoring chronic HF. Furthermore, the high index of individuality of >0.6 at time scales of one month and above suggests that results may be better referenced to a population, negating the ability to use serial changes of an individual to predict decompensation. Certainly, this appears to

be so in our cohort.

Several studies have reported on observed changes in apelin levels following various treatment modalities. In a study of eleven patients with advanced HF undergoing left ventricular assist device implantation, Chen et al demonstrated that expression of the APJ receptor was significantly reduced. In the same cohort, repeated sampling at the time of transplantation revealed significantly increased myocardial apelin and APJ receptor concentrations⁽²⁴⁷⁾, however no assessment of plasma apelin concentrations was performed. It is therefore unclear whether these findings translate into easily measurable differences in the peripheral circulation. More recently, in a group of fourteen patients undergoing CRT, Francia et al were able to demonstrate restoration of normal apelin concentrations nine months post device implantation in those deemed to be CRT 'responders'⁽²⁴⁸⁾. These changes represented a doubling of apelin, but were also documented in the single 'non-responder' despite further clinical deterioration and rising NTproBNP. Such results indicate that changes in plasma apelin concentrations do not correspond with clinical outcome. The results of my own study corroborate these findings.

Although a decrease in apelin concentration was observed in those patients experiencing a CV admission at one month, this failed to reach significance. The trend was also not observed over the three or six-month follow-up, making any conclusive interpretation of this result impossible. Overall, neither absolute nor percentage changes in apelin showed any ability to predict CV admission in stable chronic HF patients.

No studies to date have examined the effect of initiation and optimisation of evidence based medication on apelin concentrations. Previous animal studies have shown that the down-regulation of myocardial ACE2 in apelin knockout mice is reversed following the administration of angiotensin receptor blockers⁽³²⁰⁾, however no such correlation has yet to be examined in human

HF cohorts. It could, therefore, be hypothesised that apelin may have its greatest use in monitoring those patients undergoing optimisation – where it would be expected to see a rise in apelin as therapy is introduced and up titrated. Given that this study required all patients to be on optimum doses of prognostic medication, it is unknown how apelin concentrations behave under these circumstances. Moreover, despite optimisation, median concentrations in this cohort remained below that of the normal reference range, despite relatively low levels of NTproBNP.

Clearly the role of apelin in HF pathophysiology still has more questions than answers. Current evidence would suggest that apelin provides no additional use as either a diagnostic or prognostic marker and its real strength may lie in its therapeutic potential.

6.7 Conclusion

The biological variability of apelin is significantly lower than that of NTproBNP, but this does not translate into improved monitoring potential. Percentage and absolute changes in apelin are no better at predicting CV admission than those of NTproBNP in patients with stable chronic HF. These findings suggest that apelin is unlikely to be a useful biomarker for monitoring patients with stable chronic HF.

Soluble ST2 (sST2)

7.1 Introduction

Soluble ST2 (sST2) is a member of the interleukin 1 (IL-1) receptor family that has recently been identified as a novel biomarker for cardiac remodelling and fibrosis. Several studies have shown it to be a strong prognostic marker in acute/recently decompensated HF^(138, 258-261). Compared with the B-type natriuretic peptides, its release is less affected by obesity, age, atrial fibrillation, aetiology, or prior diagnosis of HF - making it an attractive candidate for monitoring. To date, three studies have provided evidence for the prognostic role of serial measures of sST2 in patients with acute HF^(259, 260, 265). The role of sST2 in chronic HF is less well defined^(257, 321), however baseline concentrations have been shown to be prognostic in this setting⁽²⁶²⁻²⁶⁴⁾.

Whilst the prognostic benefits of baseline sST2 demonstrated by these trials cannot be ignored, the aim of this present study is to examine the potential for sST2 as a monitoring tool, over and above its longer-term prognostic benefits. For the purpose of monitoring it is changes in serial sampling that are of most interest. This study is the first to examine the calculated biological variability with the ability of serial changes to predict CV admission over a six-month follow-up period in the same cohort of pharmacologically optimised stable HF patients (NYHA I-III).

7.2 Methods

This study was performed according to the methods and calculations set out in chapter 3 sections 3.1-3.2 and 3.5-3.8.

7.3 Results

7.3.1 Patient Characteristics

Characteristics of the 50 patients are described in section 4.1.

7.3.2 Biomarker Concentrations

The distributions of all results for NTproBNP and sST2 were non-parametric. Concentrations of NTproBNP and sST2 at each visit are detailed in table 22. There were no significant differences in median concentrations from baseline across the time points for either NTproBNP (one hour $p=0.874$, one month $p=0.883$, three months $p=0.144$, six months $p=0.279$) or sST2 (one hour $p=0.746$, one month $p=0.958$, three months $p=0.857$, six months $p=0.752$).

	Baseline	One Hour	One Month	Three Months	Six Months
NTproBNP	300	285	466	291	356
(ng/L)	(80.8-1282)	(90.6-1150)	(80.2-1171)	(46.5-1006)	(61.9-1469)
sST2	17.6	18.2	17.3	17.1	16.9
(µg/L)	(13.3-22.5)	(12.1-25.9)	(13.6-21.5)	(13.8-22.5)	(13.7-21.6)

Table 22: Median concentrations of NTproBNP and sST2 at each time point

BIOLOGICAL VARIABILITY

7.4.1 Analytical Coefficient of Variation (CV_a)

Mean intra-assay coefficient of variation was utilised as an estimate of overall CV_a . Using this calculation, the CV_a 's for NTproBNP and sST2 were 4.17% and 4.83% respectively.

7.4.2 Individual Coefficients of Variation, Index of Individuality and Reference Change Values

CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and sST2 at each time point are shown in table 23.

	1 Hour		1 Month		3 Months		6 Months	
	NTproBNP	sST2	NTproBNP	sST2	NTproBNP	sST2	NTproBNP	sST2
CV_i	18.47	9.99	36.75	12.02	40.98	12.23	46.02	16.41
CV_g	28	33	28	29	32	25	32	30
II	0.65	0.30	1.31	0.41	1.28	0.48	1.44	0.54
RCV (%)	52	31	103	36	114	36	128	47

Table 23: CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and sST2 at each time point

Paired t-tests were used to examine differences in CV_i across the time points. Compared with one hour CV_i , significant variability was seen across all time points for NTproBNP; one hour to one month $p=0.003$, one hour to three months $p<0.001$, and one hour to six months $p=0.003$. Variability for sST2 existed only between one hour and six months; one hour to one month $p=0.362$, one hour to three months $p=0.382$, and one hour to six months $p=0.019$.

No significant difference was demonstrated between CV_i for NTproBNP and sST2 at one hour ($p=0.076$). Significant differences were, however, observed between CV_i for NTproBNP and sST2 at all other time points; one month $p<0.001$, three months $p<0.001$ and six months $p<0.001$.

7.4.3 Individual Coefficients of Variation, Index of Individuality and Reference Change Values:

Stable Patients

CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and sST2 at each time point were re-calculated after removal of any patients reaching the primary end-point of the study. Results are shown in table 24.

	1 Hour		1 Month		3 Months		6 Months	
	NTproBNP	sST2	NTproBNP	sST2	NTproBNP	sST2	NTproBNP	sST2
CV_i	18.47	9.99	36.15	12.44	41.54	12.98	42.92	13.54
CV_g	28	33	29	30	32	36	34	32
II	0.65	0.30	1.25	0.41	1.28	0.36	1.39	0.43
RCV								
(%)	52	31	100	37	115	38	120	39

Table 24: CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and sST2 at each time point for patients not experiencing a CV admission.

Paired t-tests were used to examine differences in CV_i across the time points. After removal of patients experiencing a CV admission, variability for NTproBNP remained significant across all time points; one hour to one month $p=0.029$, one hour to three months $p=0.019$ and one hour to six months $p=0.022$. For sST2, however, no variability was now observed across any time point; one hour to one month $p=0.514$, one hour to three months $p=0.674$ and one hour to six months $p=0.238$.

No significant difference was demonstrated between CV_i for NTproBNP and sST2 at one hour ($p=0.076$). Significant differences were, however, observed between CV_i for NTproBNP and sST2 at all other time points; one month $p<0.001$, three months $p=0.003$ and six months $p=0.026$.

SERIAL MONITORING

7.5.1 Baseline correlations

Pearson correlation was carried out to evaluate the relationship of baseline NTproBNP and sST2 with several patient characteristics related to prognosis (table 25).

In keeping with previous studies on NTproBNP, baseline concentrations significantly correlated with LVEF ($p=0.001$), end diastolic volume (EDV) ($p=0.011$), duration of QRS complex on 12 lead ECG ($p=0.008$), NYHA class ($p=0.042$), creatinine ($p=0.006$) and eGFR ($p=0.012$).

sST2 significantly correlated with duration of QRS complex on 12 lead ECG ($p=0.007$). No association was observed between sST2 and any other characteristic.

	NTproBNP	sST2
NTproBNP	1.00	0.033
sST2	0.033	1.00
Age	0.251	-0.097
LVEF	-0.439**	0.258
LVEDV	0.355*	0.122
NYHA Class	0.289*	0.204
HR	-0.018	-0.247
QRS	0.372**	0.376**
Creatinine	0.382**	-0.080
eGFR	-0.352*	0.231

Table 25: Pearson correlation of baseline NTproBNP and sST2 with patient characteristics related to prognosis
(* significant at the 0.05 level, ** significant at the 0.01 level)

7.5.2 Single absolute concentrations and cardiovascular events

ROC analysis of single absolute concentrations at baseline, one month and three months showed NTproBNP to be better than sST2 at predicting CV admission over the six month study period (figures 40 and 41) (AUC 0.764; 95% CI 0.619 to 0.910; $p=0.028$, AUC 0.780; 95% CI 0.636 to 0.924; $p=0.020$ and AUC 0.714; 95% CI 0.540 to 0.889; $p=0.075$ vs. AUC 0.582; 95% CI 0.334 to 0.831; $p=0.469$, AUC 0.622; 95% CI 0.402 to 0.841; $p=0.284$ and AUC 0.576; 95% CI 0.327 to 0.825; $p=0.505$ respectively).

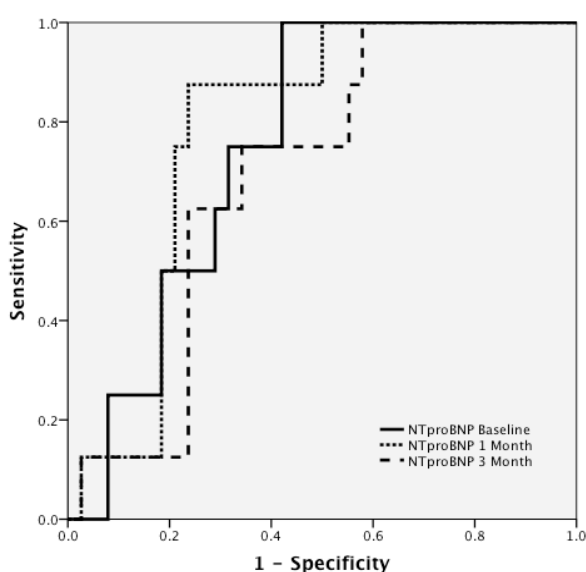


Figure 40: ROC analysis of single absolute NTproBNP concentrations and CV admission

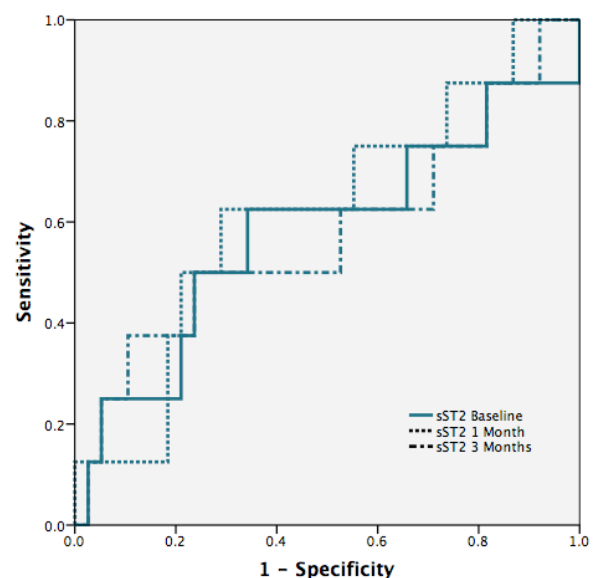


Figure 41: ROC analysis of single absolute sST2 concentrations and CV admission

7.5.3 Performance characteristics of serial sST2 as a discriminator of patient risk

The value of relative changes in both NTproBNP and sST2 were assessed by considering absolute and percentage changes from baseline to follow-up measurement.

Median (Interquartile Range (IQR)) percentage changes for the group overall and for those with and without a CV admission are shown in table 26. Differences were observed, at each time point, for both sST2 and NTproBNP between those experiencing a CV admission and those who did not. Statistical significance, however, was only demonstrated for sST2 at six months. Boxplot analysis of percentage change in sST2 and NTproBNP according to CV admission is shown in figures 42 and 43.

Time		Overall n	Overall Median % change	CV Adm (n)	CV Adm Median % change (IQR)	No CV Adm (n)	No CV Adm Median % change	p value
Baseline		50	-	-	-	-	-	-
			-		-		-	-
1 month	sST2	47	-1.9 (-12.2-15.1)	3	13.0 (-1.3-29.8)	44	-2.5 (-12.2-15.2)	0.345
	NTproBNP		-7.2 (-28.6-44.0)		13.2 (-54.4-82.9)		-8.9 (-28.5-50.4)	0.822
3 months	sST2	46	-0.25 (-15.4-13.3)	6	-5.6 (-25.2-14.9)	40	2.11 (-13.2-12.6)	0.473
	NTproBNP		-21.4 (-49.2-38.3)		-41.5 (-65.9 - -7.3)		-9.1 (-45.1-44.3)	0.811
6 months	sST2	45	0.60 (-17.8-20.5)	7	20.6 (11.3-77.3)	38	-1.5 (-18.0-17.8)	0.049
	NTproBNP		8.9 (-22.7-66.2)		39.8 (-24.6-138.9)		5.1 (-28.6-59.5)	0.571

Table 26: Comparison of median interquartile range (IQR) percentage changes for NTproBNP and sST2 overall and for those with and without a CV admission

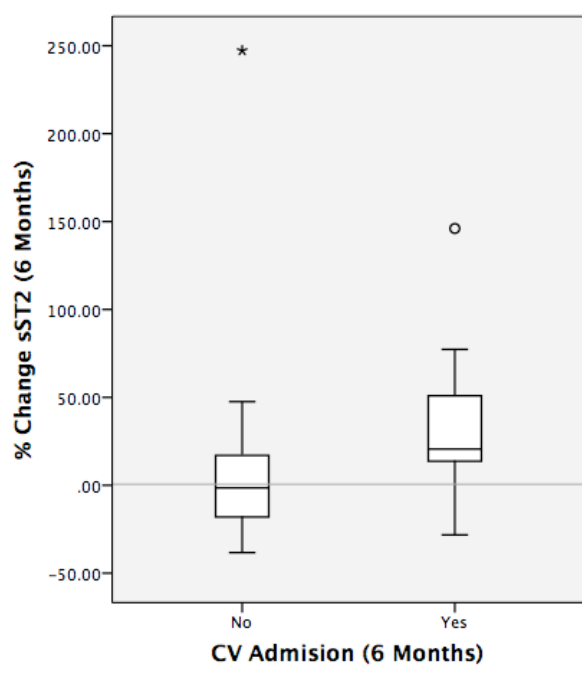


Figure 42: Box-plot analysis of % change in sST2 over six months according to CV admission status (o=outlier 1.5 x IQR, ★= outlier 3 x IQR)

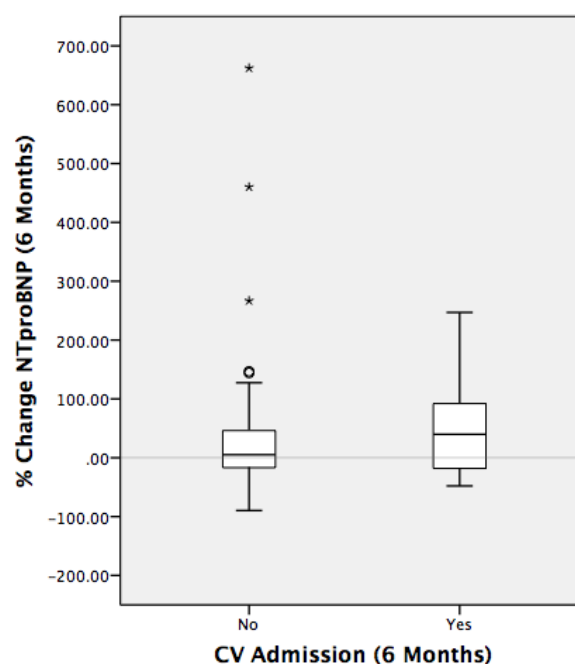


Figure 43: Box-plot analysis of % change in NTproBNP over six months according to CV admission status (o=outlier 1.5 x IQR, ★= outlier 3 x IQR)

ROC analysis of absolute changes showed sST2 was no better than NTproBNP at predicting CV admission at one month (AUC 0.455; 95% CI 0.074 to 0.835; $p=0.794$ vs. AUC 0.538; 0.133 to 0.943; $p=0.828$) (figure 44) or three months (AUC 0.379; 95% CI 0.118 to 0.640; $p=0.133$ vs. AUC 0.296; 95% CI 0.046 to 0.546; $p=0.110$) (figure 45). The AUC for absolute change in sST2 between baseline and six months was 0.734 (95% CI 0.49 to 0.97; $p=0.05$). This result only just failed to reach statistical significance for the ability of sST2 to predict CV admission over the six-month period, but was greater than that of absolute change in NTproBNP (AUC=0.579; 95% CI 0.28 to 0.88; $p=0.511$ vs. AUC 0.571; 95% CI 0.341 to 0.802; $p=0.553$) (figure 46).

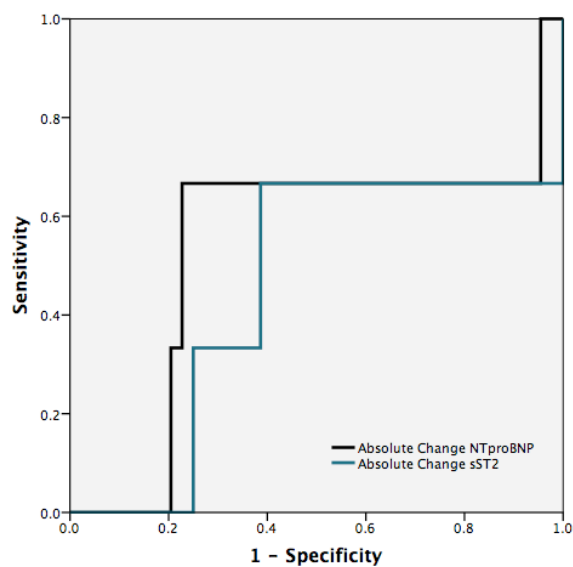


Figure 44: ROC analysis of absolute change in sST2 and NTproBNP over one month and CV admission

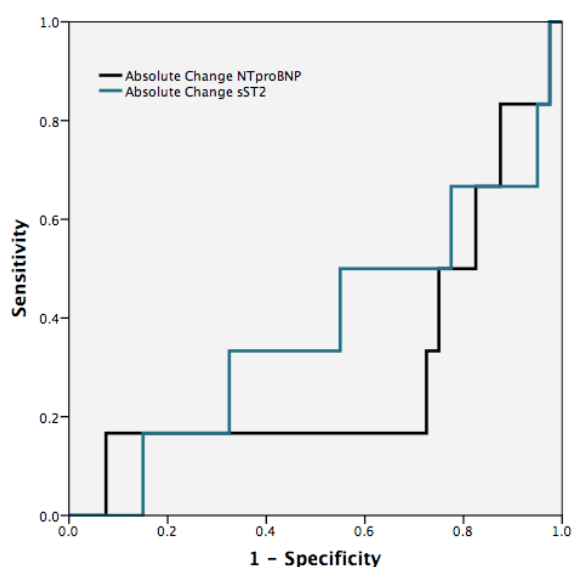


Figure 45: ROC analysis of absolute change in sST2 and NTproBNP over three months and CV admission

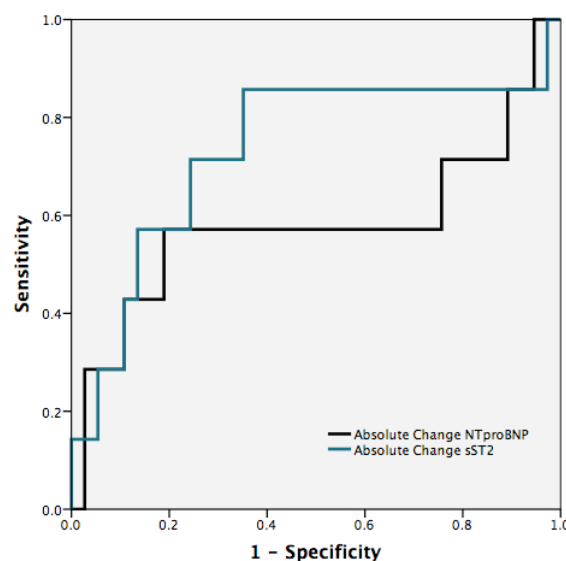


Figure 46: ROC analysis of absolute change in sST2 and NTproBNP over six months and CV admission

Similar results were observed for percentage changes in sST2 and NTproBNP, with no significant ability to predict CV admission at one month (AUC 0.455; 95% CI 0.074 to 0.835; $p=0.794$ vs. AUC 0.455; 95% CI 0.142 to 0.767; $p=0.794$) (figure 47) or three months (AUC 0.404; 95% CI 0.138 to 0.671; $p=0.453$ vs. AUC 0.325; 95% CI 0.100 to 0.550; $p=0.171$) (figure 48). The AUC for percentage change in sST2 between baseline and six months was 0.734 (95% CI 0.51 to 0.95; $p=0.05$). As for absolute change, this result only just failed to reach statistical significance, but was greater than that of percentage change in NTproBNP (AUC 0.571; 95% CI 0.34 to 0.80; $p=0.553$) (figure 49).

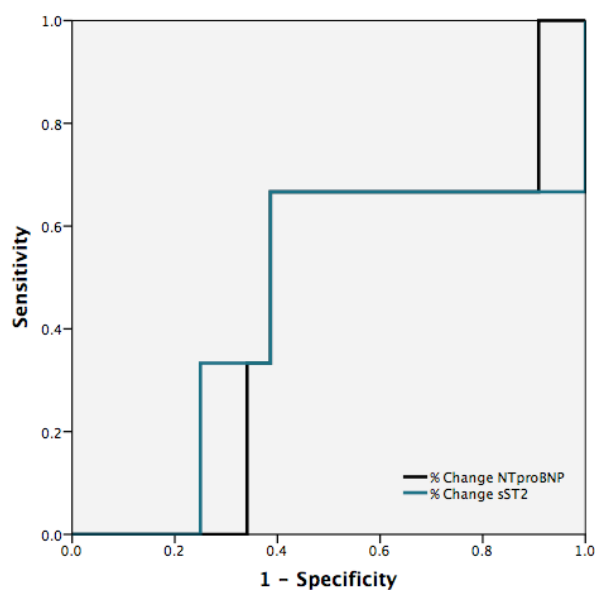


Figure 47: ROC analysis of % change in sST2 and NTproBNP over one month and CV admission

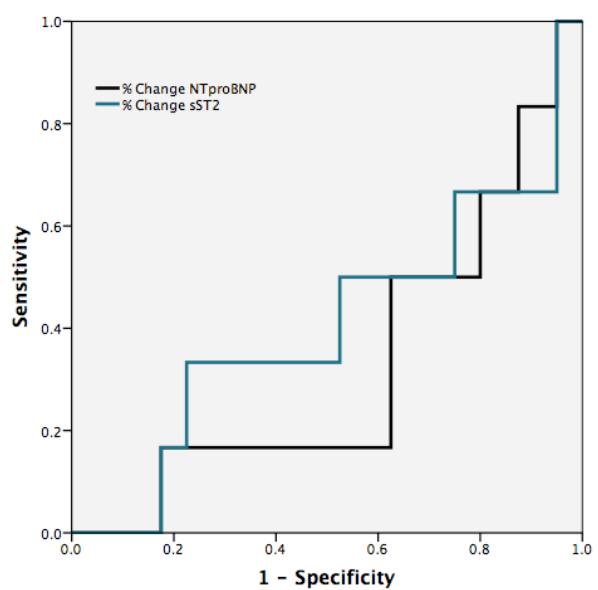


Figure 48: ROC analysis of % change in sST2 and NTproBNP over three months and CV admission

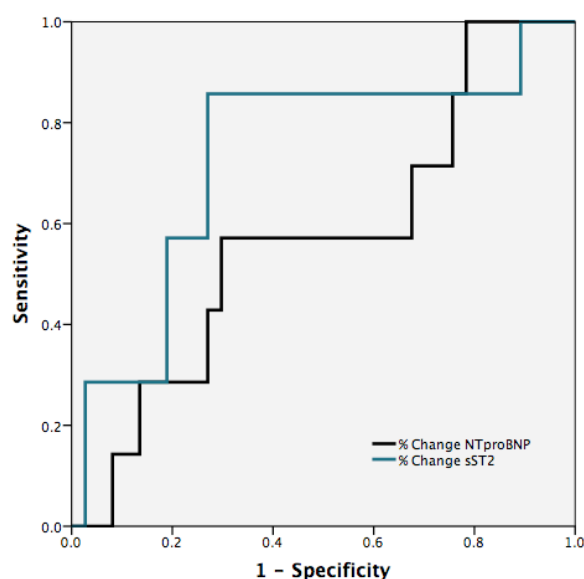


Figure 49: ROC analysis of % change in sST2 and NTproBNP over six months and CV admission

7.5.4 sST2 concentrations and renal function

Absolute sST2 concentrations showed no correlation with creatinine at either baseline, one month or three months (baseline $p=0.581$, one month $p=0.385$, three months $p=0.719$). Significant correlation was, however, observed at six months ($p=0.009$). No correlation with eGFR was observed at any time point (baseline $p=0.107$, one month $p=0.128$, three months $p=0.407$, six months $p=0.121$).

Insufficient events occurred at one month to allow meaningful ROC analysis at this time point. No significant relationship between absolute or percentage changes in sST2 and worsening renal function (increase in creatinine $\geq 25\%$) was demonstrated at three months (AUC 0.597; CI 0.356 to 0.838; $p=0.578$ and AUC 0.612; CI 0.390 to 0.835; $p=0.519$ respectively). Absolute and percentage change in sST2 and worsening renal function at six months, however, showed clear differences and revealed a significant ability of sST2 to detect this change compared to NTproBNP (AUC 0.913; 95% CI 0.814 to 1.0; $p=0.007$ vs. AUC 0.563; 95% CI 0.215 to 0.910; $p=0.683$) (figure 50) and (AUC 0.894, 95% CI 0.78-1.0; $p=0.010$ vs. AUC 0.506, 95% CI 0.15-0.86; $p=0.967$) (figure 51), respectively.

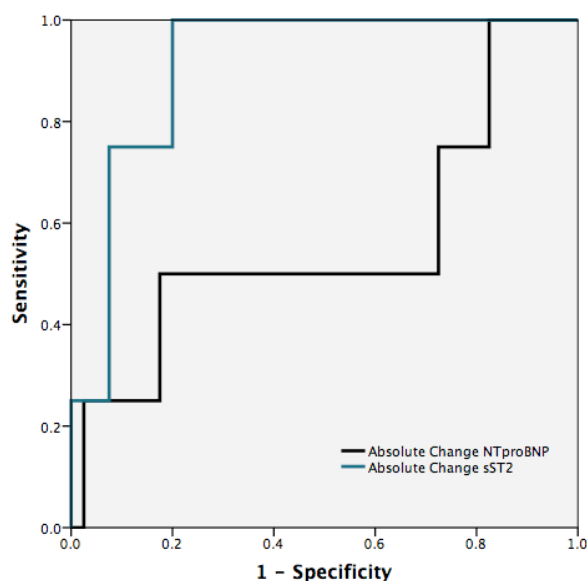


Figure 50: ROC analysis of absolute change in sST2 and NTproBNP over six months and renal function

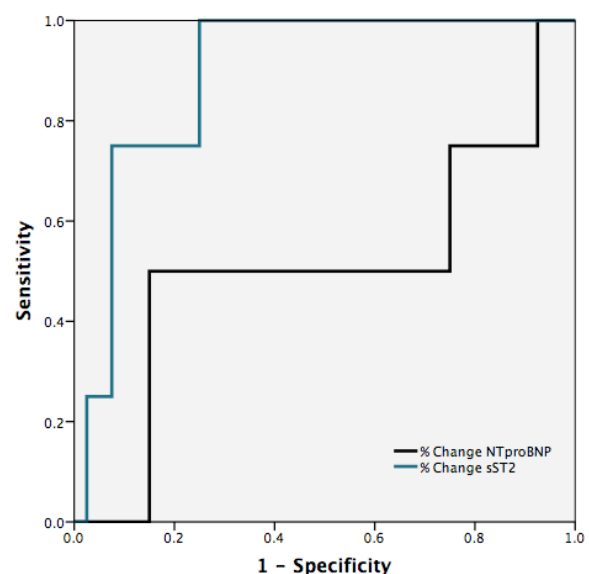


Figure 51: ROC analysis of % change in sST2 and NTproBNP over six months and worsening renal function

7.6 Discussion

Compared with NTproBNP, sST2 had significantly lower biological variability at all time points except one hour. Across time points, sST2 demonstrated significant differences in calculated RCV between one hour and six months, however no significant differences were observed between any other time points. Clearly this could have implications for serial monitoring, however it is unlikely that any monitoring policy would include sampling at time points as short as one hour. Although not assessed in this study, as with apelin, it would be necessary to evaluate BV and RCV at a minimum of two weeks, the current recommended follow-up time post discharge following acute decompensation in the UK⁽³⁰²⁾, in order to establish reliable BV components. Moreover, after removal of the results of patients who experienced a CV admission, this significant difference was no longer demonstrated. Given that a CV admission would expect to be related to changes in sST2 concentrations and therefore the variability both between and within individuals, it could also be expected that this would affect the corresponding coefficients of variability and therefore the RCV. Removal of such variability and the subsequent changes in results strongly support the hypothesis that sST2 may be better than NTproBNP for serial monitoring purposes.

Further support for these findings is the low Index of Individuality of sST2, indicating significant differences between individuals relative to the population. Consequently, single concentrations cannot be effectively compared to a population-based reference range - with serial sampling providing more meaningful comparisons. In the case of sST2, the calculated index is higher than that previously reported in healthy individuals⁽²⁶⁶⁾. This appears to be the results of lower calculated CV_g – and therefore lower variation between individuals. The reason for this lower variability within the chronic HF population compared to controls is somewhat surprising and should be validated in further studies. Despite these higher values, however, overall the calculated results remain below that which would suggest single measures would be more useful and therefore should not impact on the potential usefulness of sST2 as a monitoring tool.

Despite these promising biological variability results, serial and absolute changes in sST2 in the same cohort were statistically no better than NTproBNP at predicting CV admission over a six-month period. Results at six-months, however, almost reached statistical significance on ROC analysis. Moreover, analysis of median percentage sST2 changes at six months did demonstrate a statistically significant difference between those experiencing a CV admission and those who did not.

To date, there are limited data on the use of serial sST2 in chronic HF. A previous sub-study analysis of the Prospective Randomized Amlodipine Survival Evaluation 2 (PRAISE-2) trial demonstrated that, in 161 patients with NYHA class III-IV non-ischaemic HF, changes in sST2 over a two-week period were associated with an increased risk of death or transplantation⁽²⁵⁷⁾.

As in this study, however, no such association was observed with single baseline measurements. One possible explanation for such findings may relate to the shorter time intervals and chronicity of symptoms examined. Whilst sST2 is linked with myocardial hypertrophy and fibrosis, both of these are late manifestations of cardiac disease, occurring over several months. By examining patients with stable chronic disease, it could be argued that the baseline concentrations of sST2 measured reflect 'background levels' for these individuals, which would then be expected to rise in accordance with acute decompensation – resulting in a change in serial measurements. Indeed, results from my own study support this hypothesis, showing that median baseline sST2 levels were in fact within the reference range for the assay utilised in this study. Moreover, given that QRS width is associated with degree of fibrosis⁽³²²⁾, this may also go some way to explain the correlation with baseline sST2 observed in this study; identifying those with higher 'background levels' of sST2 and possibly higher degrees of underlying fibrosis. Whilst such individuals may indeed be at increased long-term risk, a follow-up of only six-months in this study may be insufficient to fully appreciate the deleterious effects of either already present or increasing levels of fibrosis and

would be difficult to detect in the small numbers recruited. Future studies could incorporate the use of cardiac magnetic resonance imaging to further classify the degree of fibrosis in the cohort under examination and how this relates to sST2 levels.

It should be remembered, however, that sST2 is not merely a marker of fibrosis but is also associated with immune modulation and inflammatory responses^(250, 252, 323). In the shorter-term, changes in sST2 may therefore reflect different underlying processes such as inflammation or, as demonstrated in this study, renal dysfunction. sST2 has previously been shown to be associated with disease severity in chronic kidney disease⁽³²⁴⁾ but no studies have examined the relationship between serial sST2 and changes in renal function. The relationship with worsening renal function observed in this study requires further clarification. Whilst this may simply reflect changes in renal function observed with worsening HF or intercurrent infection/inflammation, previous studies have found no influence of renal function on the prognostic value of sST2⁽³²⁵⁾. Larger studies are required in order to validate this finding and further expand our understanding of the relationship between sST2 and renal function.

7.7 Conclusion

The biological variability of sST2 is significantly lower than that of NTproBNP, but this may not translate into improved monitoring potential, at least in the short term. Percentage and absolute changes in sST2 were unable to predict CV admission or worsening renal function in patients with pharmacologically optimised chronic HF than NTproBNP at times scales of less than six months, but only just failed to reach statistical significance at the six-month endpoint. Given the association of sST2 with IL-33, this may reflect the progression of myocardial fibrosis as an important mechanism in the progression of chronic HF and the impact of inflammatory responses on both cardiac and renal function. Larger studies are required to fully validate its potential for monitoring purposes.

Galectin-3

8.1 Introduction

Galectin-3 is a soluble β -galactosidase-binding lectin implicated in the regulation of cardiac fibrosis and remodelling. Concentrations in plasma are strongly related to outcome and are independent predictors of mortality in a wide range of clinical presentations of HF^(243, 275-277, 279-281).

Several studies have examined the use of serial plasma galectin-3 measurements in HF patients^(136, 137, 282). Results of these studies indicate that increases in galectin-3 concentrations over time are independent predictors of both mortality and HF hospitalisation in patients either recently hospitalised with decompensation or with known CAD and stable angina. The use of serial measurements in routine clinical practice, however, has yet to be validated and no studies have assessed the use of serial sampling in patients with chronic HF, who are stable and on optimal medical therapy.

To date, only two published studies have explored the biological variability of galectin-3 in human subjects^(266, 283). Only one has compared this with NTproBNP in the same cohort, with promising results⁽²⁶⁶⁾. No studies have examined the biological variability of galectin-3 in the medically treated chronic HF population.

In this study, I examine the biological variability of galectin-3 and present novel findings regarding the use of repeated measures, specifically in those with stable chronic HF with full pharmacological optimisation.

8.2 Methods

This study was performed according to the methods and calculations set out in chapter 3 sections 3.1-3.2 and 3.5-3.8.

8.3 Results

8.3.1 Patient Characteristics

Characteristics of the 50 patients are described in section 4.1.

8.3.2 Biomarker Concentrations

The distribution of all results for NTproBNP and galectin-3 were non-parametric. Concentrations of NTproBNP and galectin-3 at each visit are detailed in table 27. There were no significant differences in median concentrations from baseline across the time points for either NTproBNP (one hour $p=0.874$, one month $p=0.883$, three months $p=0.144$, six months $p=0.279$) or galectin-3 (one hour $p=0.619$, one month $p=0.568$, three months $p=0.519$, six months $p=0.542$).

	Baseline	One Hour	One Month	Three Months	Six Months
NTproBNP	300	285	466	291	356
(ng/L)	(80.8-1282)	(90.6-1150)	(80.2-1171)	(46.5-1006)	(61.9-1469)
Galectin-3	7.9	7.7	8.2	8.2	8.1
(µg/L)	(6.9-9.3)	(6.1-9.8)	(6.2-9.6)	(6.2-9.5)	(6.3-9.8)

Table 27: Median concentrations of NTproBNP and galectin-3 at each time point

BIOLOGICAL VARIABILITY

8.4.1 Analytical Coefficient of Variation (CV_a)

Mean intra-assay coefficient of variation was utilised as an estimate of overall CV_a . Using this calculation, the CV_a 's for NTproBNP and galectin-3 were 4.17% and 3.87% respectively.

8.4.2 Individual Coefficients of Variation, Index of Individuality and Reference Change Values

CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and galectin-3 at each time point are shown in table 28.

	1 Hour		1 Month		3 Months		6 Months	
	NTproBNP	Gal-3	NTproBNP	Gal-3	NTproBNP	Gal-3	NTproBNP	Gal-3
CV_i	18.47	12.73	36.75	13.98	40.98	14.48	46.02	18.13
CV_g	28	20	28	20	32	18	32	20
II	0.65	0.63	1.31	0.69	1.28	0.80	1.44	0.90
RCV								
(%)	52	37	103	40	114	42	128	51

Table 28: CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and galectin-3 at each time point

Paired t-tests were used to examine differences in CV_i across the time points. Compared with one hour CV_i , significant variability was seen across all time points for NTproBNP; one hour to one month $p=0.003$, one hour to three months $p<0.001$, and one hour to six months $p=0.003$. Variability for galectin existed only between one hour and six months; one hour to one month $p=0.393$, one hour to three months $p=0.254$, and one hour to six months $p=0.012$.

No significant difference was demonstrated between CV_i for NTproBNP and galectin-3 at one hour ($p=0.095$). Significant differences were, however, observed between CV_i for NTproBNP and galectin-3 at all other points; one month $p<0.001$, three months $p<0.001$ and six months $p=0.001$.

8.4.3 Individual Coefficients of Variation, Index of Individuality and Reference Change Values:

Stable Patients

CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and galectin-3 at each time point were re-calculated after removal of any patients reaching the primary end-point of the study. Results are shown in table 29.

	1 Hour		1 Month		3 Months		6 Months	
	NTproBNP	Gal-3	NTproBNP	Gal-3	NTproBNP	Gal-3	NTproBNP	Gal-3
CV_i	18.47	12.73	36.15	12.33	41.54	12.47	42.92	13.26
CV_g	28	20	29	21	32	20	34	20
II	0.65	0.63	1.25	0.58	1.28	0.62	1.39	0.66
RCV (%)	52	37	100	34	115	36	120	38

Table 29: CV_g , CV_i , index of individuality and corresponding reference change values for NTproBNP and galectin-3 at each time point for patients not experiencing a CV admission.

Paired t-tests were used to examine differences in CV_i across the time points. After removal of patients experiencing a CV admission, variability for NTproBNP remained significant across all time points; one hour to one month $p=0.029$, one hour to three months $p=0.019$ and one hour to six months $p=0.022$. For galectin-3, however, no variability was now observed across any time point; one hour to one month $p=0.393$, one hour to three months $p=0.975$ and one hour to six months $p=0.278$.

No significant difference was demonstrated between CV_i for NTproBNP and galectin-3 at one hour ($p=0.095$). Significant differences were, however, observed between CV_i for NTproBNP and galectin-3 at all other time points; one month $p<0.001$, three months $p=0.002$ and six months $p=0.014$.

SERIAL MONITORING

8.5.1 Baseline correlations

Pearson correlation was carried out to evaluate the relationship of baseline NTproBNP and galectin-3 with several patient characteristics related to prognosis (table 30). In keeping with previous studies on NTproBNP, baseline concentrations significantly correlated with LVEF ($p=0.001$), end diastolic volume (EDV) ($p=0.011$), duration of QRS complex on 12 lead ECG ($p=0.008$), NYHA class ($p=0.042$), creatinine ($p=0.006$) and eGFR ($p=0.012$). Although baseline galectin-3 correlated with baseline NTproBNP ($p=0.042$), of the other parameters, it only significantly correlated with creatinine ($p=0.008$) and eGFR ($p=0.006$).

	NTproBNP	Galectin-3
NTproBNP	1.00	0.289 [*]
Galectin-3	0.289 [*]	1.00
Age	0.251	0.104
LVEF	-0.439 ^{**}	-0.093
LVEDV	0.355 [*]	0.049
NYHA Class	0.289 [*]	0.145
HR	-0.018	0.239
QRS	0.372 ^{**}	0.014
Creatinine	0.382 ^{**}	0.371 ^{**}
eGFR	-0.352 [*]	-0.381 ^{**}

Table 30: Pearson correlation of baseline NTproBNP and galectin-3 with patient characteristics related to prognosis (* significant at the 0.05 level, ** significant at the 0.01 level)

8.5.2 Single absolute concentrations and cardiovascular events

ROC analysis of single absolute concentrations at baseline, one month and three months showed NTproBNP to be better than galectin-3 at predicting CV admission over the six month study period

(figures 52 and 53) (AUC 0.764; 95% CI 0.619 to 0.910; $p=0.028$, AUC 0.780; 95% CI 0.636 to 0.924; $p=0.020$ and AUC 0.714; 95% CI 0.540 to 0.889; $p=0.075$ vs. AUC 0.408; 95% CI 0.169 to 0.647; $p=0.417$, AUC 0.520; 95% CI 0.293 to 0.747; $p=0.862$ and AUC 0.520; 95% CI 0.296 to 0.744; $p=0.862$ respectively).

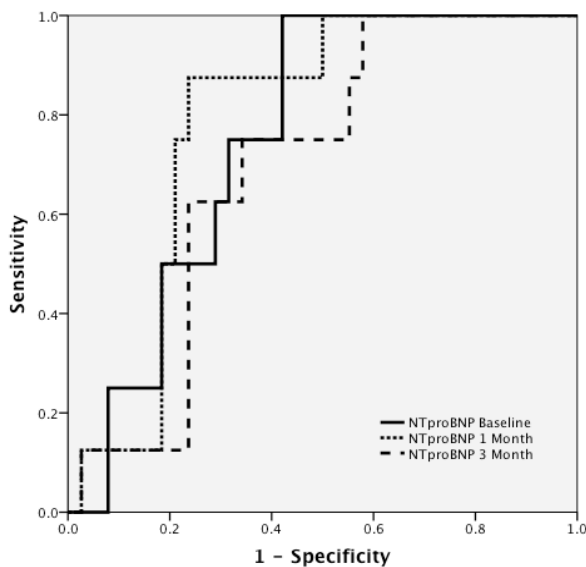


Figure 52: ROC analysis of single absolute NTproBNP concentrations and CV admission

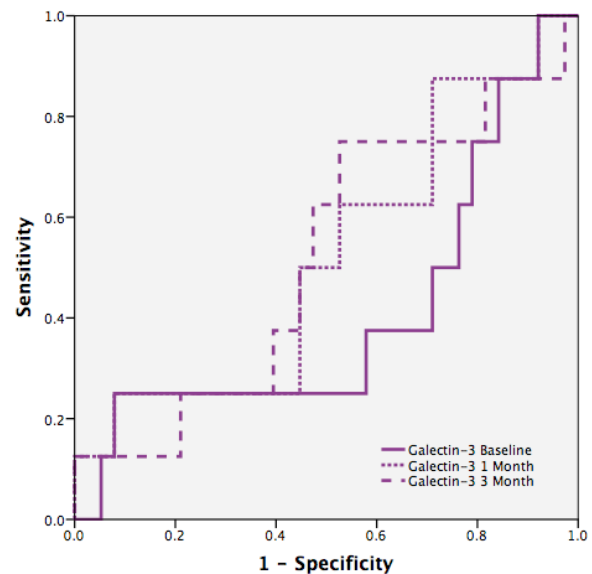


Figure 53: ROC analysis of single absolute galectin-3 concentrations and CV admission

8.5.3 Performance characteristics of serial Galectin-3 as a discriminator of patient risk

The value of relative changes in both NTproBNP and galectin-3 were assessed by considering absolute and percentage changes from baseline to follow-up measurement.

Median (Interquartile Range (IQR)) percentage changes for the group overall and for those with and without a CV admission are shown in table 31. Differences were observed, at each time point, for both galectin-3 and NTproBNP. Statistical significance, however, was only demonstrated for galectin-3 at six months. Box-plot analysis of these findings show a clear separation in galectin-3 concentrations between those with and those without a CV admission (figure 54). No significant difference was observed for changes in NTproBNP (figure 55).

Time		Overall n	Overall Median % change	CV Adm (n)	CV Adm Median % change (IQR)	No CV Adm (n)	No CV Adm Median % change	p value
Baseline		50	-	-	-	-	-	-
			-		-		-	
1 month	Galectin-3	47	3.6 (-8.4-10.0)	3	7.9 (-3.8-7.9)	44	2.9 (-9.5-9.9)	0.548
	NTproBNP		-7.2 (-28.6-44.0)		13.2 (-54.4-82.9)		-8.9 (-28.5-50.4)	0.822
3 months	Galectin-3	46	0.72 (-11.0-19.8)	6	11.5 (-15.7-26.5)	40	-1.5 (-10.2-18.5)	0.644
	NTproBNP		-21.4 (-49.2-38.3)		-41.5 (-65.9 - -7.3)		-9.1 (-45.1-44.3)	0.811
6 months	Galectin-3	45	4.1 (-12.3-23.2)	7	28.3 (15.1-41.8)	38	0.55 (-17.7-13.5)	0.008
	NTproBNP		8.9 (-22.7-66.2)		39.8 (-24.6-138.9)		5.1 (-28.6-59.5)	0.571

Table 31: Comparison of median interquartile range (IQR) percentage changes for NTproBNP and galectin-3 overall and for those with and without a CV admission

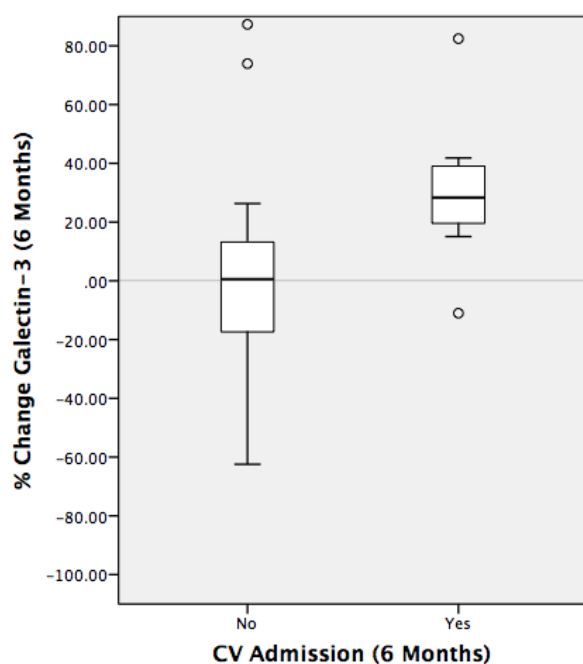


Figure 54: Box-plot analysis of % change galectin-3 over 6 months according to CV admission status (o=outlier 1.5 x IQR, ★= outlier 3 x IQR)

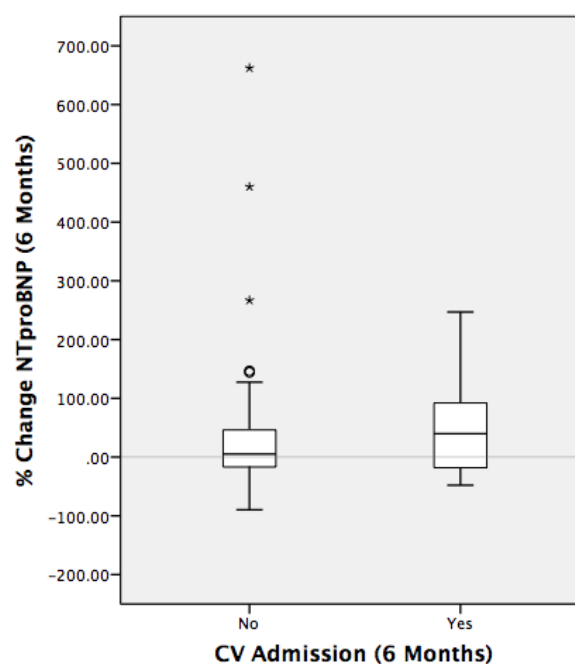


Figure 55: Box-plot analysis of % change in NTproBNP over 6 months according to CV admission status (o=outlier 1.5 x IQR, ★= outlier 3 x IQR)

ROC analysis of absolute changes showed galectin-3 was better than NTproBNP at predicting CV admission at one month (AUC 0.598; 95% CI 0.396 to 0.801; $p=0.572$ vs. AUC 0.538; 95% CI 0.133 to 0.943; $p=0.828$) (figure 56), and three months (AUC 0.567; 95% CI 0.305 to 0.828; $p=0.602$ vs. AUC 0.296; 95% CI 0.046 to 0.546; $p=0.110$) (figure 57), but only reached statistical significance at six months (AUC 0.807; 95% CI 0.608 to 1.00; $p=0.011$ vs. AUC 0.579; 95% CI 0.282 to 0.877; $p=0.511$) (figure 58).

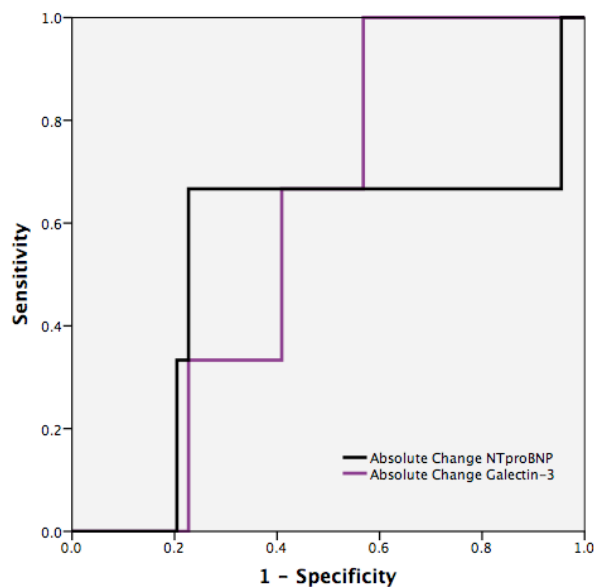


Figure 56: ROC analysis of absolute changes in galectin-3 and NTproBNP over one month and CV admission

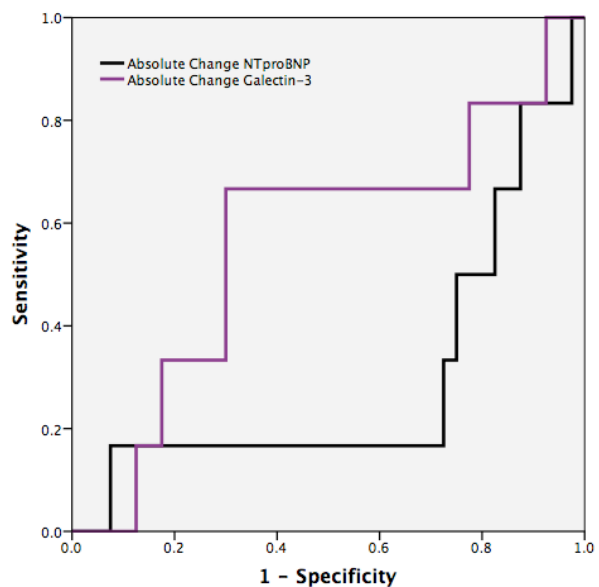


Figure 57: ROC analysis of absolute changes in galectin-3 and NTproBNP over three months and CV admission

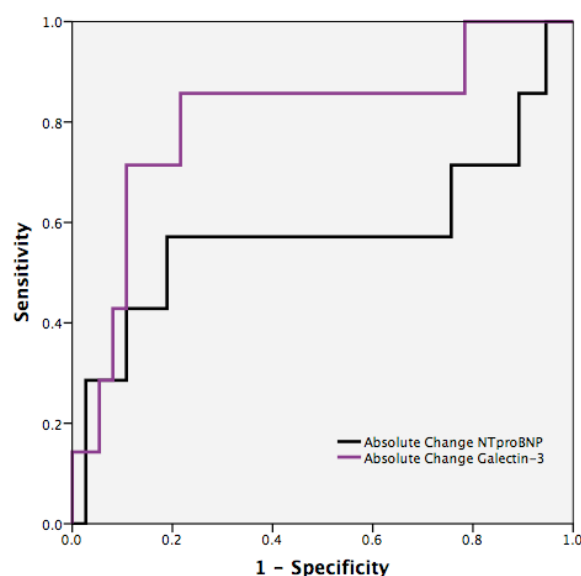


Figure 58: ROC analysis of absolute changes in galectin-3 and NTproBNP over six months and CV admission

Similar results were demonstrated for percentage change in galectin-3. At both one month (AUC 0.614; 95% CI 0.386 to 0.841; $p=0.514$ vs. AUC 0.455; 95% CI 0.142 to 0.767; $p=0.794$) (figure 59), and three months (AUC 0.563; 95% CI 0.305 to 0.820; $p=0.625$ vs. AUC 0.325; 95% CI 0.100 to 0.550; $p=0.171$) (figure 60), galectin-3 was better than NTproBNP at predicting CV admission. Once again statistical significance was only demonstrated at six months (AUC 0.803; 95% CI 0.617 to 0.989; $p=0.012$ vs. AUC 0.571; 95% CI 0.341 to 0.802; $p=0.553$) (figure 61).

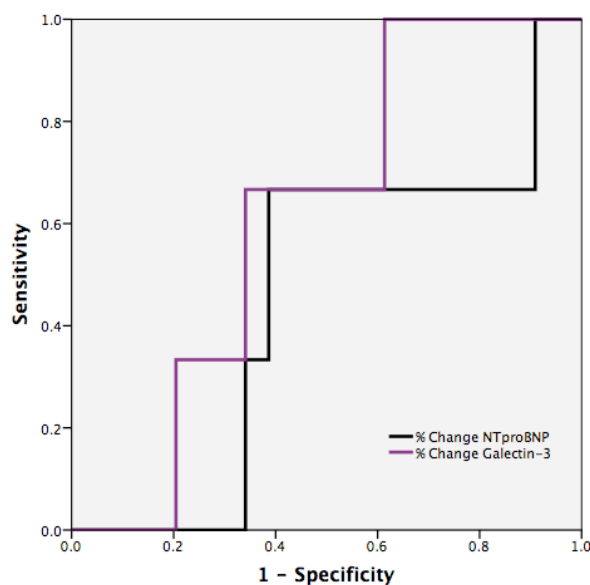


Figure 59: ROC analysis of % change in galectin-3 and NTproBNP over one month and CV admission

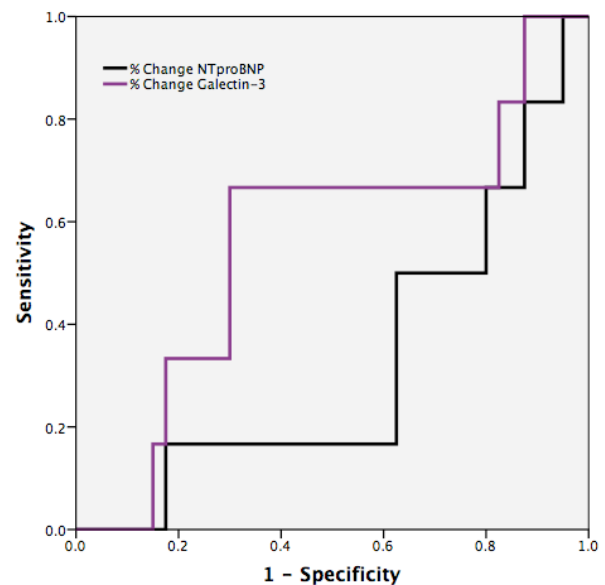


Figure 60: ROC analysis of % change in galectin-3 and NTproBNP over three months and CV admission

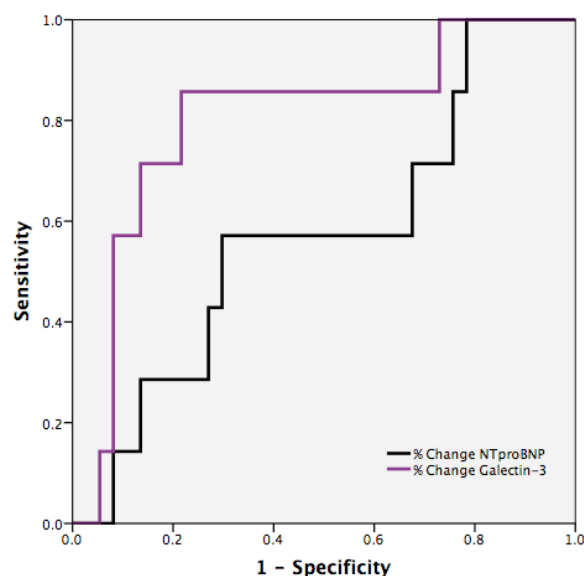


Figure 61: ROC analysis of % change in galectin-3 and NTproBNP over six months and CV admission

Further ROC analysis found no relationship between CV admission and change in galectin-3 >15% (AUC 0.500; 95% CI 0.176 to 0.824; p=1.00).

8.5.4 Galectin-3 concentrations and renal function

At each time point, single galectin-3 concentrations were significantly correlated with both creatinine (baseline p=0.008, one month p<0.001, three months p=0.048, six months p=0.001) and eGFR (baseline p=0.006, one month p=0.001, three months p=0.038, six months p=0.016).

Insufficient events occurred at one month to allow meaningful ROC analysis at this time point. No significant relationship between absolute or percentage changes and worsening renal function was demonstrated at three; AUC 0.457; 95% CI 0.031 to 0.884; p=0.807 and AUC 0.488; 95% CI 0.022 to 0.955; p=0.947, or six months; AUC 0.598, 95% CI 0.199 to 0.996, p=0.523 and AUC 0.591, 95% CI 0.199 to 0.984, p=0.550 respectively.

8.6 Discussion

Compared with NTproBNP, galectin-3 had significantly lower biological variability at all time points except one hour. Across time points, galectin-3 demonstrated significant differences in calculated RCV between one hour and six months, however no significant differences were observed between any other time points. Clearly this could have implications for serial monitoring, however it is unlikely that any monitoring policy would include sampling at time points as short as one hour. As with both apelin and sST2, it would be necessary to evaluate BV and RCV at a minimum of two weeks, the current recommended follow-up time post discharge following acute decompensation in the UK⁽³⁰²⁾, in order to establish reliable BV components. Moreover, after removal of the results of patients who experienced a CV admission, this significant difference was no longer demonstrated. Given that a CV admission would expect to be related to changes in galectin-3 concentrations and therefore the variability both between and within individuals, it could also be

expected that this would affect the corresponding coefficients of variability and therefore the RCV. Removal of such variability and the subsequent changes in results strongly support the hypothesis that galectin-3 may be better than NTproBNP for serial monitoring purposes.

Factors which may offset this potential, however, include the finding of an index of individuality of >0.6 at all time points. This high II suggests that results may be better referenced to a population, negating the ability to use serial changes of an individual to predict decompensation. These results are relatively lower but in keeping with previous results by Wu et al⁽²⁶⁶⁾, who reported hourly and two month II of galectin-3 of 1.00 and 1.01 respectively. Despite this, however, my results demonstrate that both absolute and percentage changes in galectin-3 were significantly better than NTproBNP at predicting CV admission at six months in the same cohort of patients. It is unclear what the explanation for this may be. It is possible that, given the optimised, stable nature of the cohort, the reference population in this study were sufficiently similar that referencing to the individual also reflected referencing to the cohort. Clearly, the complexities of biological variability are such that interpretation cannot be reduced to single variables alone.

Although earlier trials looking at changes in galectin-3 and prognosis initially indicated that changes in galectin-3 over six months did not add prognostic information to the baseline concentration alone⁽²⁷⁸⁾, more recent studies have disputed this finding⁽¹³⁶⁾. This study provides added evidence to the argument that such changes are indeed related with prognosis. Specifically, it demonstrates that, even in a stable, fully optimised cohort, not only do galectin-3 concentrations change over time, but that at six months, these changes are more closely related to the outcome measure of CV admission than paired changes in the natriuretic peptide NTproBNP. Furthermore, this study corroborates earlier findings that serial measurements add prognostic information significantly beyond that provided by single measures alone^(136, 137).

Consistent with the findings of Motiwala *et al*⁽¹³⁶⁾, serial measurements at six months appear to be optimal for providing additional prognostic information than measurements at any earlier time point. In contrast to these previous studies, however, I was unable to demonstrate any significant relationship between galectin-3 changes of >15% and CV admission.

Galectin-3 concentrations have previously been shown to correlate with renal function. In this study I demonstrate that, although single absolute concentrations at each time point correlated with both creatinine and eGFR, no such correlation existed for either absolute or percentage change. Moreover, serial measurements were not shown to predict worsening renal function over the six-month study period. Such findings indicate that changes in galectin-3 are not significantly related to changes in renal function, either as a result of decompensated heart failure *per se*, or as a result of changes in diuretic therapy. They may therefore more accurately reflect underlying pathological hypertrophic/fibrotic processes that are, as yet, not fully targeted by evidence-based therapies. This may also go some way to explain why, to date, there is no evidence to show that galectin-3 concentrations are significantly affected by prognostically indicated medications or CRT.

8.7 Conclusion

The biological variability of galectin-3 is significantly lower than that of NTproBNP. Moreover, percentage and absolute changes in galectin-3 are better predictors of CV admission than those of NTproBNP in patients with stable chronic HF. These findings suggest that, compared with NTproBNP, galectin-3 may be a better biomarker for monitoring patients with stable chronic HF. The use of serial measurements may allow safer discharge of patients for monitoring by community teams/general practitioners. Questions remain, however, as to how prognostic therapies effect galectin-3 levels. Further larger prospective randomised controlled trials examining the use of galectin-3 for monitoring are required to further validate these findings and clarify the impact of currently available therapies on galectin-3 triggered intervention.

Multi-Marker Analysis

9.1 Introduction

Multi-markers strategies are increasingly used as clinical tools in the risk stratification of patients presenting with chest pain and acute coronary syndromes⁽³²⁶⁻³²⁸⁾ and for predicting future risk of cardiovascular disease^(329, 330). In contrast, the field of multi-marker evaluation in HF remains in its infancy and, of the studies that have evaluated such strategies, most have focused primarily on the prediction of prognosis^(243, 261, 331-333).

Several general concepts inform the type of multi-marker strategies most likely to be clinically useful. Fundamentally, markers that reflect distinct biological processes are more likely to provide incremental clinical benefit than markers that relate to the same general physiologic phenomenon. Optimal methods for choosing which individual biomarkers to include in a multi-marker panel have, however, not been well established. Historically those biomarkers with the strongest independent statistical association with disease or outcome have been chosen, with some emphasis on selecting biomarkers that capture distinct aspects of the disease process⁽³³⁴⁾.

In a study of prognosis in patients presenting acutely with dyspnoea, Rehman et al⁽³³⁵⁾ hypothesised that simultaneous assessment of pathophysiologically diverse biomarkers would provide complementary prognostic information, irrespective of the eventual causal diagnosis. In this, they examined several of the novel biomarkers presented in this thesis, including sST2, galectin-3 and apelin, as well as NTproBNP. Using data from the PRIDE study⁽¹⁶⁹⁾, ROC analysis was used to identify candidate markers for prediction of mortality at one year – with a minimum AUC of 0.7 selected as the cut off for inclusion in the multi-marker model. In addition, those identified were tested for statistical significance for independent prognostic ability and only if both conditions were met, were the markers included in the final model. Using this strategy, neither galectin-3 or apelin demonstrated sufficient significance and were therefore excluded, with the final model consisting of NTproBNP, sST2, C-reactive protein (CRP), haemoglobin and blood urea nitrogen. 60% of their

cohort was diagnosed with HF as the cause of their acute dyspnoea. The one-year risk of death increased in proportion to the increase in multi-marker scores, with mortality rates of 2.0%, 7.8%, 22.3%, 29.3%, and 57.6%, corresponding to scores of 1, 2, 3, 4, and 5 respectively.

Similar studies have examined the added benefits of several novel biomarkers to both established clinical prognostic markers, including anaemia, CRP and troponin, as well as the B-type natriuretic peptides^(332, 333). Of the novel biomarkers in this present study, promising results have been demonstrated for the addition of all except apelin. In a study of 599 patients with acute dyspnoea, the combination of galectin-3 to NTproBNP was the best predictor of 60-day mortality in subjects with acute HF⁽²⁴³⁾. In the same study, apelin was not found to be of any diagnostic or prognostic benefit. In a much larger, international trial of over 5,000 patients, Lassus et al demonstrated that the addition of NT-proBNP, CRP, MR-proADM and sST2 to the clinical model, resulted in significantly higher c-statistics for the prediction of 30-day mortality⁽²⁶¹⁾.

To date, no published studies have examined the use of a multi-marker approach for the monitoring of patients with chronic HF.

9.2 Methods

This study was performed according to the methods set out in chapter 3 sections 3.1-3.6 and 3.8.

9.3 Results

9.3.1 Patient Characteristics

Characteristics of the 50 patients are described in Chapter 4, section 4.1.

9.3.2 Baseline correlations

Pearson correlation was carried out to evaluate the relationship between baseline concentrations

of the four novel biomarkers and NTproBNP (table 32).

As demonstrated previously, baseline MRproADM was highly significantly correlated with NTproBNP ($p<0.001$), with galectin-3 also showing a correlation, but at a much lower significance ($p=0.042$). No correlation was observed between NTproBNP and apelin or sST2.

Of the novel biomarkers, apelin was shown to correlate with MRproADM ($p=0.008$). No other correlation was demonstrated.

	NTproBNP	MRproADM	Apelin	sST2	Galectin-3
NTproBNP	1.00	0.486**	0.244	0.033	0.289*
MRproADM	0.486**	1.00	0.373**	0.129	0.044
Apelin	0.244	0.373**	1.00	0.094	0.018
sST2	0.033	0.129	0.094	1.00	-0.077
Galectin-3	0.289*	0.044	0.018	-0.077	1.00

Table 32: Baseline biomarker correlations (* significant at the 0.05 level, ** significant at the 0.01 level)

9.3.3 Identification of candidate biomarkers

Using the same criteria as described by Rehman et al⁽³³⁵⁾, ROC analysis of the percentage change in the novel biomarkers demonstrated only sST2 and galectin-3 to have an AUC of >0.70 and therefore be eligible for inclusion in a 'multi-marker' approach (figure 62). In order to determine if changes in any clinical markers would be potential candidates for a multi-marker approach, further ROC analyses of the percentage change of several markers of HF prognosis, including haemoglobin, urea, creatinine, eGFR and bilirubin was also carried out. All failed to show an AUC greater than 0.7 (figure 63).

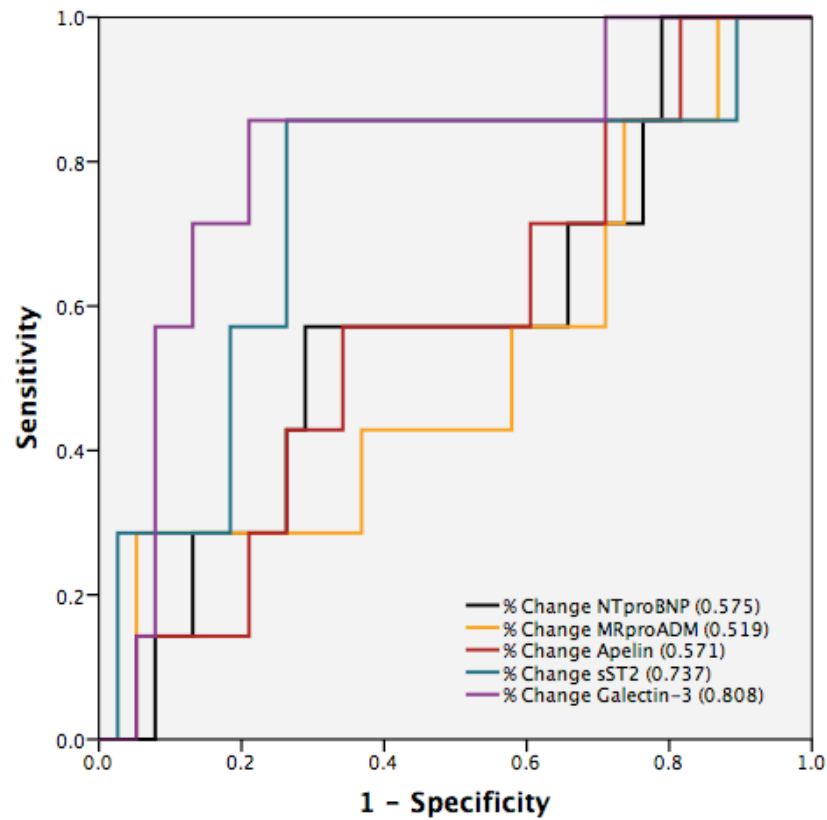


Figure 62: ROC analysis of % change of all studied biomarkers over six months and CV admission (AUC)

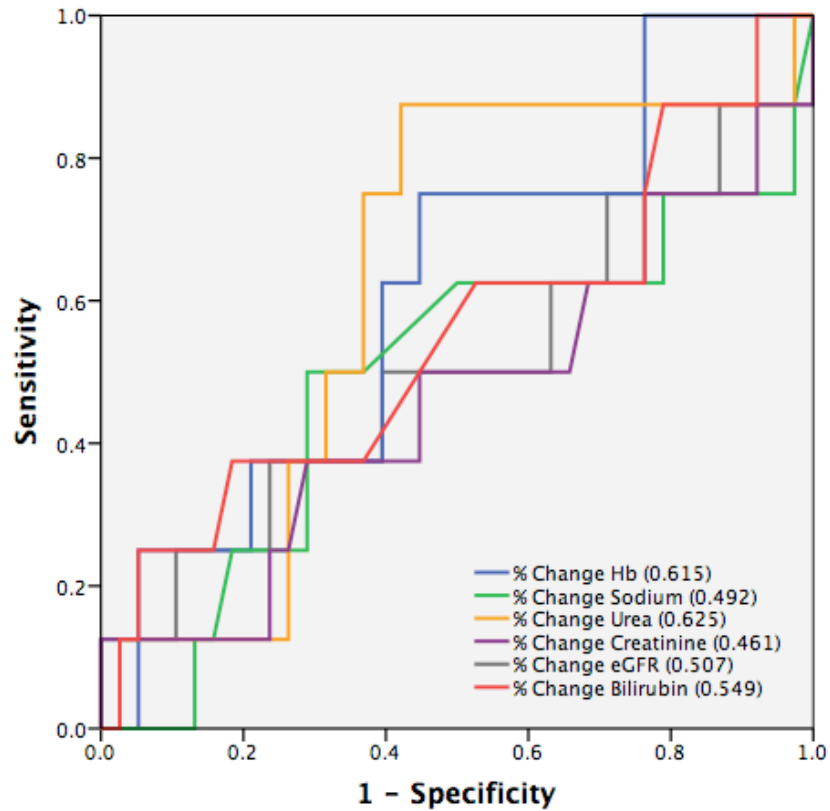


Figure 63: ROC analysis of % change in clinical biomarkers over six months and CV admission (AUC)

9.3.4 Multi-marker analysis

Despite attempts to perform a Cox proportional hazards analysis, unfortunately too few events occurred to allow any meaningful statistical analysis to take place. With only eight events over the six-month period, and one set of bloods missing, attempts to statistically prove a percentage change in any marker as independent for predicting CV admission were not possible. Consequently, no further statistical analysis of a multi-marker model was performed.

Examining the spread of patients according to CV admission and whether they exhibited a rise in 0,1,2,3,4 or 5 of the biomarkers above the median percentage change for the cohort confirms no obvious association of a higher score with CV admission (figure 64).

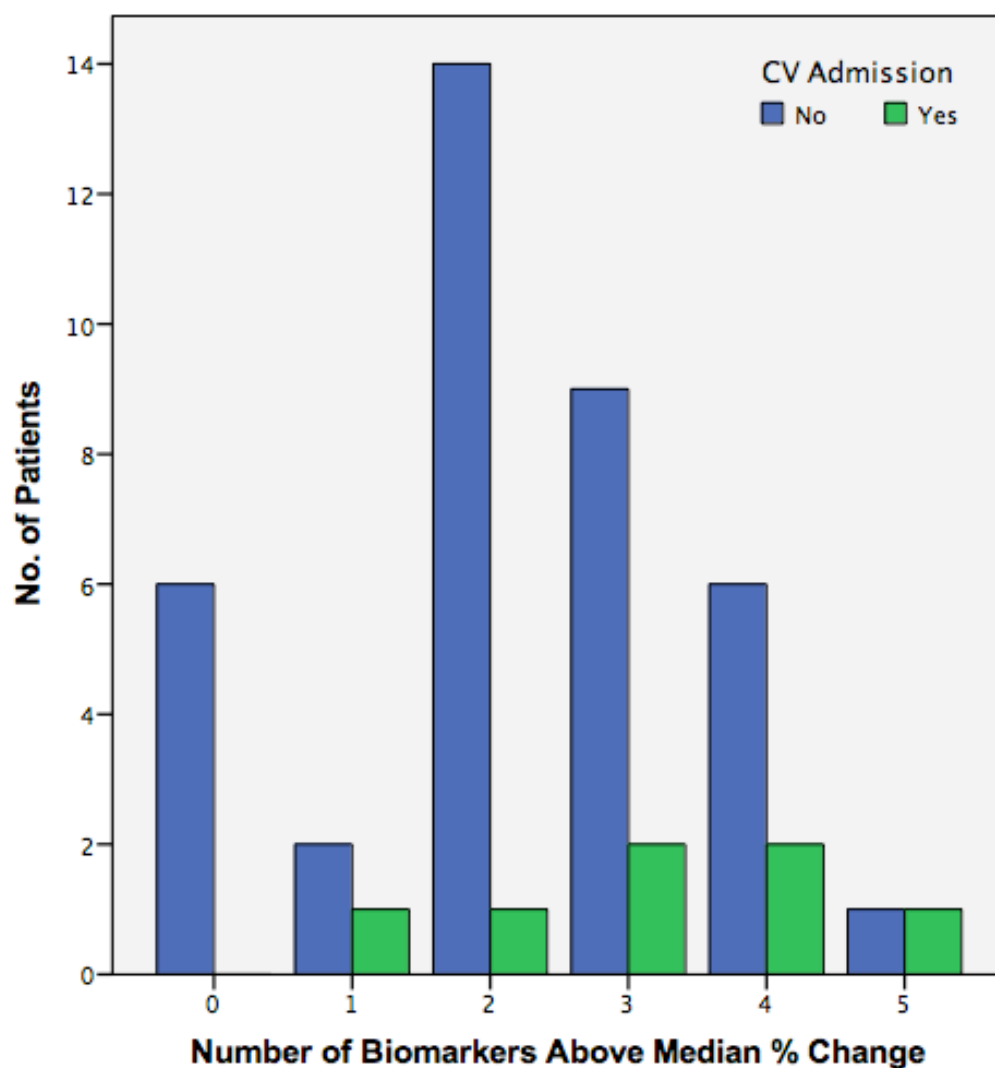


Figure 64: CV admission according to number of biomarkers with % change above the median

Of the eight patients experiencing an event over the six-month study period, blood samples were available for only seven, further degrading any statistical power. Examining these patients with respect to percentage changes in biomarker concentrations compared to the median percentage change over six-months does reveal a trend towards sST2 and galectin-3 being potentially useful (table 33). Of the four novel biomarkers, percentage changes in these two markers were raised above the median in six of the seven patients (86%). This compared with NTproBNP, MRproADM and apelin all of which demonstrated almost equal numbers of patients with changes above and below the median change for the entire cohort.

Patient	NTproBNP	MRproADM	Apelin	sST2	Galectin-3
1	↓	↑	↓	↑	↑
2	Six-month sample lost after arrival in lab				
3	↓	↓	↓	↑	↑
4	↑	↑	↓	↑	↑
5	↓	↓	↑	↑	↑
6	↓	↓	↓	↓	↑
7	↑	↑	↑	↑	↑
8	↑	↓	↑	↑	↑

Table 33: Change in biomarkers above or below the median % change over six months in patients with a CV admission

Combined with the results of ROC analysis, these results represent a signal that both galectin-3 and sST2 may be candidates for a multi-marker approach to monitoring of chronic HF. When examined in the context of the entire cohort, however, the numbers are once again too few to demonstrate any obvious trend towards percentage changes in these two markers above the median change being associated with CV admission (figure 65).

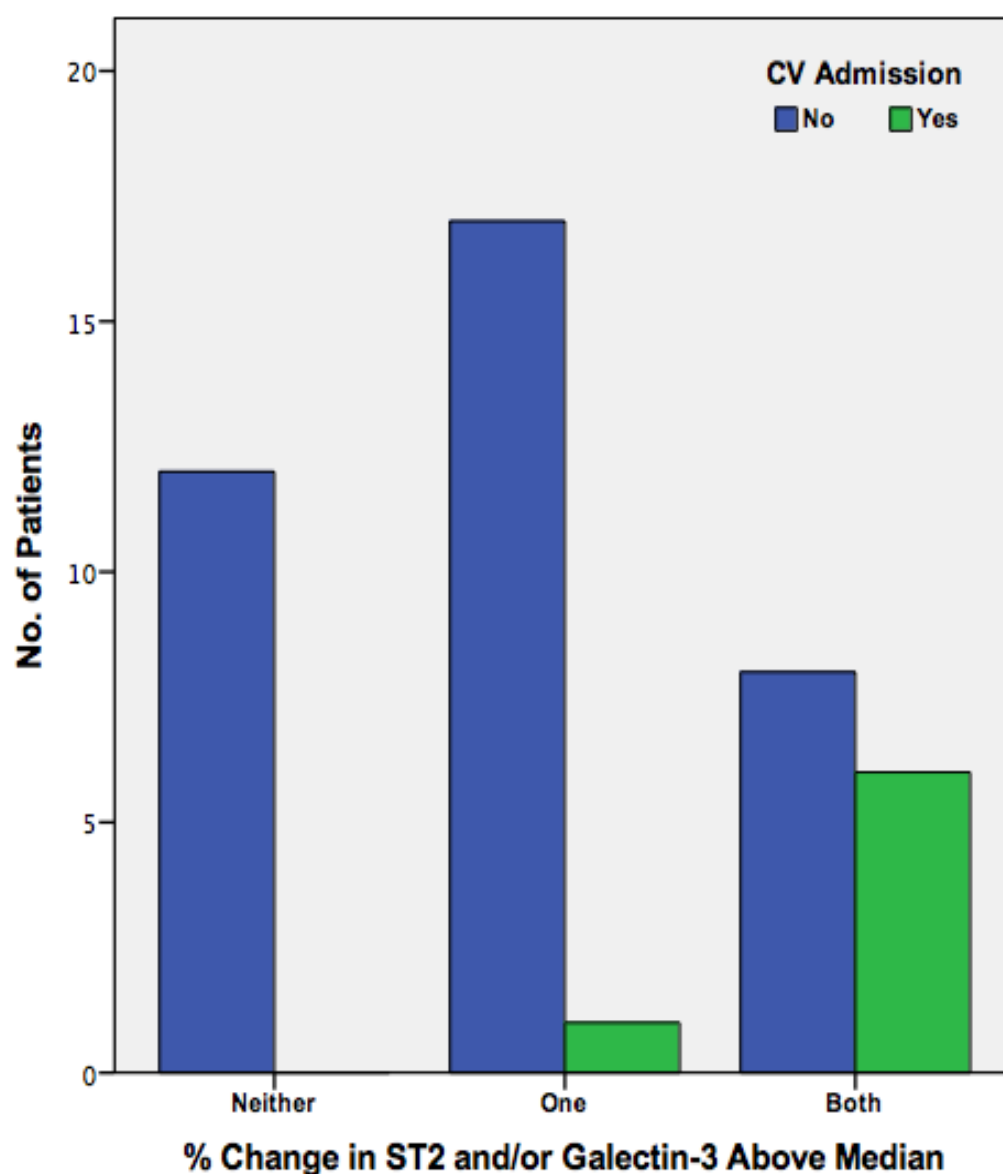


Figure 65: CV admission according to % change in sST2 and/or galectin-3 above the median

Moreover, it should be noted that, in order to allow any type of analysis, results were pooled across the study period of six months, rather than being analysed in relation to admission time point. Details of admission relative to recruitment have been described previously and can be found in section 4.1, table 10. Interestingly, it should be noted that those with the highest number of increases in biomarkers above the median percentage change over the six months were also those with the longest times between recruitment and admission (table 34).

Patient	NTproBNP	MRproADM	Apelin	sST2	Galectin-3	Time from recruitment to admission (days)
1	↓	↑	↓	↑	↑	41
2	Six-month sample lost after arrival in lab					147
3	↓	↓	↓	↑	↑	23
4	↑	↑	↓	↑	↑	68
5	↓	↓	↑	↑	↑	26
6	↓	↓	↓	↓	↑	13
7	↑	↑	↑	↑	↑	156
8	↑	↓	↑	↑	↑	44

Table 34: Change in biomarkers above or below the median % change over six months in patients with a CV admission relative to time of admission

9.4 Discussion

The evaluation of multi-marker strategies is becoming an increasingly popular field in HF research. To date most have focused on the prognostic ability of such approaches, with no published data on the potential of multi-marker strategies for monitoring chronic HF.

Although one of the original objectives of this research was to evaluate a multi-marker approach, the small sample size, follow-up period and subsequent event rate resulted in too few results to allow statistically meaningful analyses. The results presented here indicate a signal towards both sST2 and galectin-3 being useful for this purpose, but this can neither be verified either statistically or clinically in the current cohort.

9.5 Conclusion

Future development of multi-marker approaches has the potential to improve HF care along the entire spectrum of disease, by improving screening, simplifying diagnosis, clarifying prognosis, and tailoring treatment and follow-up strategies. No single biomarker can accomplish all these goals in isolation, suggesting that multi-markers approaches are likely to become increasingly prevalent in HF care. Although this study was unable to shed significant light on the benefits of such approaches in the monitoring of chronic HF, it is clear that the potential remains and that both galectin-3 and sST2 may be candidate markers in such strategies. Further, larger studies are required to clarify this potential.

LIMITATIONS OF THE STUDY

Several limitations of the study that may affect the interpretation of results are acknowledged.

10.1 Sample size

This study involved a small sample size and was conducted at a single tertiary care centre. Overall, the small sample size represents the most significant limitation of the study. As a result, it is underpowered with respect to the number of events and therefore to detect statistical significance at time points earlier than at the six months demonstrated, or conduct multivariate or hazard analyses. In contrast to previous studies, I was unable to demonstrate correlation with prognosis by baseline concentrations or any specific thresholds of change. Again, this is likely due to the sample size and event rate limiting statistical significance. Indeed, findings such as the reduction in MRproADM in those experiencing a CV admission at one month and three months compared with those who did not have a CV admission can only logically be explained by the small sample size and the presence of outliers, rather than a true reflection of MRproADM changes in the disease state.

This study, however, was designed as a pilot study to stimulate interest in the most promising biomarkers. In this sense, this body of work has achieved its aim. Moreover, the sample size reflects the stringent nature of the entry criteria to ensure recruitment was restricted to those truly pharmacologically optimised and stable at baseline. This fact is further corroborated by the relatively low median biomarker concentrations observed at baseline and subsequently at each time point. In addition, the restricted numbers provided the advantage of having all patients reviewed at each time point by a single clinician and allowed all subjects to be followed closely.

10.2 Study design

10.2.1 Study Population

The design of this study required patients to exhibit stability in symptoms for at least four weeks prior to enrolment, to be on optimum tolerated HF therapy and have the ability to attend multiple

follow-up sessions. As a result, this cohort was comprised mostly of those with mild to moderate HF symptoms. Risk estimates in this population may be different to those with more severe disease and further studies are warranted to validate the results in those cohorts.

In order to improve the assessment of functional capacity, future work could incorporate more objective measures such as a six-minute walk test. Previous studies have demonstrated that six-minute walk distance is an independent predictor of hospital admission in patients with chronic HF⁽³³⁶⁻³³⁸⁾. Separating subjects according to the results of a six-minute walk test, rather than the arguably subjective NYHA classification, may have improved the strength of the study and enabled correlations between these results and biomarker concentrations.

10.2.2 Study endpoint

As previously discussed, one of the main limitations of this study relates to the sample size recruited. In addition to the innate difficulties in statistical power a small sample size portends, the resulting limitation in capturing sufficient events over a relatively short time period meant that several endpoints were necessary.

As outlined in section 3.1, a composite endpoint of CV admission, defined as an admission due to decompensated HF, arrhythmia or acute coronary syndrome, was deemed most suitable to allow meaningful statistical analysis. Although none of the study participants died during the study, it is acknowledged that mortality should have been accounted for and that an endpoint of CV admission and/or death, both cardiovascular and all-cause, would have been more robust and meaningful for the cohort of patients studied. It is suggested that future work include mortality as an outcome of interest.

10.2.3 Follow-up period

Although a six-month study period was sufficient for analysis of biological variability across the biomarkers of interest, it is acknowledged that the short follow-up period negatively impacted on the serial monitoring aspect of the study. Whilst patients with end-stage HF are highly likely to experience a CV related admission over a six-month period, this is less likely in those with milder symptoms. Given the population of interest, the monitoring aspect of this study would have benefitted from a significantly longer follow-up period, with the ability to identify outcomes of interest over a longer period increasing event rates and thereby increasing statistical power. An extended follow-up period is recommended in future studies.

10.3 Single samples

Only single samples were collected at each time point. Clinical chemistry practice has suggested that although duplicate samples result in a reduction of CV_a , this will result in double the cost of reagents and is only useful if the CV_a is >50% of the CV_i . Under these conditions, it is estimated that the assay imprecision will add only 10% to the biological variability⁽²⁶⁶⁾. Results of percentage CV_a of CV_i for each of the biomarkers in this study for all patients and for those not experiencing a CV admission are shown in tables 34 and 35.

	Percentage CV_a of CV_i			
	One hour	One Month	Three Months	Six Months
NTproBNP	23	11	10	9
MRproADM	35	37	35	25
Apelin	52	38	37	35
sST2	48	40	39	29
Galectin-3	30	28	27	21

Table 35: Percentage CV_a of CV_i for NTproBNP, MRproADM, apelin, sST2 and galectin-3

	Percentage CV_a of CV_i			
	One hour	One Month	Three Months	Six Months
NTproBNP	23	12	10	10
MRproADM	35	39	39	41
Apelin	52	37	34	35
sST2	48	39	37	36
Galectin-3	30	31	31	29

Table 36: Percentage CV_a of CV_i for NTproBNP, MRproADM, apelin, sST2 and galectin-3 for patients not experiencing a CV admission

It could be argued, therefore, that with a CV_a of >50% the CV_i of apelin at one hour, assay imprecision could add more than ten percent to the calculated biological variability. In these circumstances, the RCV at one hour would be higher (31%) but still in keeping with the results seen at one, three and six months. In fact, the CV_i at one hour would need to increase by a further 35% in order to reach the same level as that seen at one month. Regardless of this error, therefore, it remains that the variability of apelin remains substantially less than that of NTproBNP.

10.4 Biomarker Assays

Measurement of biomarker concentrations can be performed via a variety of techniques. Although the majority of assays for a particular marker employ the same technique, assays may exist to differing parts of the peptide and indeed to different isoforms. Moreover, different companies will each develop its own assay with inherent differences to that produced by other companies. From the aspect of biological variability, it is important to understand if calculated variations are comparable regardless of the technique or assay utilised.

10.4.1 MRproADM

Although assays to MRproADM have been available since 2005⁽³³⁹⁾, the first fully automated assay, developed by B.R.A.H.M.S was not formally evaluated until 2009⁽³⁴⁰⁾. Since this time several ELISA based assays have become commercially available for the research market. The majority of large trials assessing the use of MRproADM in HF populations have, however, used the original KRYPTOR system developed by B.R.A.H.M.S.^(221, 223-225, 288, 297, 299-301). Results from this study should therefore be valid for application to the majority of currently reported trials of MRproADM.

10.4.2 Apelin

Methods for apelin detection include enzyme immunoassays (EIAs) and radioimmunoassays (RIAs)^(341, 342). Most assays use antibodies to target the conserved C-terminal domain in these peptides, effectively measuring the total amount of apelin present. To characterize the specific forms of apelin present, gel filtration or high-performance liquid chromatography separation approaches coupled with apelin-like immunoreactivity detection have been used routinely. Using these approaches, the circulating concentrations for apelin show substantial disparity from a few picograms per milliliter (pg/ml) to several nanograms per milliliter (ng/ml)^(291, 316, 343).

More recently, mass spectrometry (MS) approaches, because of their high selectivity, have proved to be effective alternative methods for apelin quantification and characterization^(344, 345). In one study, the apelin concentrations for the same samples measured using a modified MS-based method and a widely-used EIA method were compared. The apelin concentrations measured using EIA were demonstrated to be much higher than the concentrations determined by MS⁽³⁴⁶⁾, corroborating previous observations reported by Mesmin and coworkers⁽³⁴⁴⁾. These disparate results require additional studies to understand this discrepancy in detail. It has been proposed that higher concentrations detected by EIA may be a result of measuring apelin immunoreactivity, with antibodies known to react with several apelin isoforms. It is thought that several of these

isoforms may not be readily detected by the MS-based methods, which are thought to reliably detect only the known biologically active forms of apelin. No studies have looked at the impact of these differing assay techniques on biological variability or whether such calculations would be comparable across them in either the healthy or disease state.

10.4.3 sST2

Currently there are three commercially available assays to sST2; the MBL assay, the Presage assay and the R&D assay. Whilst the majority of studies looking at acute HF have employed the Presage assay, studies looking at sST2 in chronic HF have used a variety of all three. Mueller et al⁽³⁴⁷⁾ performed a comparison of plasma concentrations by the three assays and found considerable differences between concentrations obtained. Results between the methods are, therefore, not directly comparable. They were not, however, able to demonstrate any superiority of one assay over the others.

Although such differences will impart changes to the CV_a , CV_i should be consistent regardless of the assay utilised. To date, there are no comparative studies assessing the BV of sST2 in chronic HF. Previous studies looking at the natriuretic peptides have, however, demonstrated similar BV and RCV across both healthy individuals and those with chronic HF. In support of my own findings, Wu et al⁽²⁶⁶⁾ recently examined the biological variability of sST2 in healthy individuals, using the Presage assay. In this they demonstrated a CV_i of 11% and RCV of 30% for sST2 at two months, results that are comparable with the CV_i of 12% and RCV of 36% observed at both one month and three months in this study.

10.4.4 Galectin-3

Similarly, there are five commercially available assays to galectin-3. Most studies have used the BGM ELISA kit, as the only FDA approved assay for measuring galectin-3. Although utilising the

same technique as the BGM assay, the R&D standard curve is substantially different from the BGM ELISA. Results from such studies are therefore not directly comparable to those presented in this study. As with sST2, there is no evidence to suggest superiority of any one assay over the others and CV_i should be consistent regardless of the assay utilised. To date, there are no comparative studies assessing the BV of galectin-3 in chronic HF. Wu et al⁽²⁶⁶⁾ recently examined the biological variability of galectin-3 in healthy individuals, using the BGM ELISA assay. In this they demonstrated a CV_i of 16% and 20% and RCV of 39% and 61% for galectin-3 at one hour and two months respectively. These results are comparable with the findings of this study at one hour with CV_i 13% and RCV 37%, but not at one month (CV_i 14%; RCV 40%) or three months (CV_i 14.5%; RCV 42%). Whether such differences are reflective of the cohort examined or the assay utilised is unclear.

DISCUSSION

Heart failure is a serious health condition, imparting significant morbidity and mortality to its sufferers. Worldwide 17–45% of patients admitted to hospital with HF die within 1 year of admission and the majority die within 5 years of admission⁽¹⁷⁾. In terms of total healthcare expenditure, it accounts for about 1–3% of that in most developed countries, including those of North America⁽³⁴⁸⁾, Western Europe⁽³⁴⁹⁾ and Latin America⁽³⁵⁰⁾. The majority of this burden comes as a result of the often lengthy and repeated hospital stays that are typically required^(349, 351). Whilst such hospital readmissions can improve survival rates among patients with worsening HF, outpatient management and avoidance of admission is undoubtedly preferable and a more efficient use of resources⁽³⁵²⁾.

Risk stratification and identification of patients with stable chronic HF who are likely to decompensate is, however, imprecise. In the UK, fear of discharging optimised patients to general practice where adherence may not be maintained has previously deterred those in secondary and tertiary care from this option, with inevitable consequences on available hospital outpatient resources. Results from both the NorthStar⁽³⁵³⁾ and COACH-2⁽³⁵⁴⁾ trials have indicated that those who are pharmacologically optimised may indeed be safe to discharge to general practice. Questions remain, however, as to how best to monitor such patients, particularly those with evidence of persistent LV dysfunction.

Despite several studies, the use of natriuretic peptides, BNP and NTproBNP, to monitor and guide treatment of patients with chronic HF is as yet unproven, with conflicting results on harder clinical outcome measures of mortality and morbidity^(129, 130, 132-135, 181-187, 192, 355). Such results have been attributed, at least in part, to the high biological variability exhibited by the natriuretic peptides in both health and disease^(188, 190, 191, 356).

The aim of this thesis was to assess the potential of four novel biomarkers of HF to monitor

pharmacologically optimised patients, both in terms of their biological variability and how this translates to the ability for changes in serial measurements to predict CV admission over a six-month period. Results were compared with those of NTproBNP.

Analysis of biological variability found that all four novel biomarkers had significantly less variability than NTproBNP. Assuming monitoring ability is based, at least in part, on biological variability, these results suggest that all four could be better than NTproBNP for monitoring purposes.

Based on biological variability alone, apelin appeared to be the most promising candidate, with low inter- and intra-individual variability and subsequent reference change values. These findings were, offset, however, by the index of individuality. Subsequently, when changes in concentration were examined over the six-month period, apelin showed no ability to predict CV admission in this cohort of patients.

Both MRproADM and sST2 produced similar, overlapping results. MRproADM demonstrated a slightly higher degree of inter-individual variation, and sST2 showed a slightly higher index of individuality, albeit still within the range of acceptability for comparing results within an individual rather than to a population. Both biomarkers, however, exhibited low intra-individual variability and reference change values in the order of 30-50% over the six-month study period. Interestingly, despite these similarities, when changes in concentration of MRproADM and sST2 were examined for their ability to predict decompensation in the form of CV admission, there was a clear difference in performance. In this respect, changes in sST2 showed substantially better ability to predict admission compared to MRproADM. This cannot be solely explained by the slightly higher inter-individual variation, as changes examined in this respect were intra-individual. Such differences indicate that the ability of a biomarker to be used for monitoring purposes does not lie solely in its properties associated with biological variability.

This finding is further corroborated by the results of galectin-3. Despite similar intra-individual variation and reference change values to both MRproADM and sST2, galectin-3 exhibited high index of individuality. Indeed, this was in the order of that seen with NTproBNP and, as with apelin, should result in poor ability to reflect changes within an individual as compared to the population. In fact, results from the serial monitoring aspect of the study revealed changes in concentrations of galectin-3 within an individual to be the overall best predictor of CV admission, demonstrating better results than sST2 for this purpose.

Similarly, differences in biomarker monitoring suitability were observed when worsening renal function was examined. Despite its ability to predict CV admission *and* significant baseline correlations with creatinine and eGFR, changes in galectin-3 demonstrated no ability to predict changes in renal function. On the other hand, sST2, showed no correlation with either creatinine or eGFR, yet changes in concentrations were reflective of changes in renal function. Moreover, MRproADM correlated with both creatinine and eGFR *and* demonstrated an ability for changes in concentration to reflect changes in renal function.

No correlation was found between worsening renal function and CV admission over the six-month time period. Thus, although worsening renal function has a known prognostic relationship with chronic HF, admissions in this cohort were not driven by such changes - potentially explaining the differences observed between the biomarkers. Like chronic HF, chronic kidney disease is a multifactorial disorder, occurring in the context of multiple co-morbid conditions, many of which are related to inflammation and inflammatory responses. MRproADM, apelin, sST2 and galectin-3 have all previously been shown to be associated with disease severity in chronic kidney disease^(228, 239, 241, 305, 324, 357-363). Variations in renal function across the time points measured could therefore be postulated to influence the calculated biological variability. This study was insufficiently powered to account for such confounding variables, however, repeated calculations performed after the

removal of any patients exhibiting a >25% change in creatinine did not result in a significant change in median CV_i or RCV for either MRproADM, sST2 or galectin-3.

It is proposed, therefore, that the ability of serial changes in a biomarker to be used for monitoring chronic HF is less dependent on the biological variability, but rather depends more on the pathophysiological process being measured, and the association of any marker with that process. For example, the natriuretic peptides, MRproADM and apelin are all markers associated, to some degree, with myocyte stretch. In this study, both NTproBNP and apelin correlated significantly with MRproADM at baseline and all three were found to have no ability for changes in concentrations to predict CV admission over the study period. Moreover, both galectin-3 and sST2 are associated with myocyte hypertrophy, with changes in both showing significant ability to predict CV admission.

No studies, however, have specifically assessed the degree to which concentrations of the novel biomarkers change as a result of prognostically indicated therapy in the outpatient setting. Clearly such information is required before any calculation of BV or RCV can be used meaningfully in this way. Moreover, there are few studies examining biomarker concentrations and variability in different disease states. Indeed, the findings of an RCV of MRproADM of 112% at one week in the post-operative OLT population by Miguel et al⁽²²⁸⁾ indicate that specific disease states may also pose an important variable on the extent of biological variability of any particular marker. Such interactions would be an important consideration in any monitoring situation. It is also accepted that CV admission may be driven by several different factors and using this as an endpoint is likely to reflect several different pathological processes. It is proposed, however, that myocyte stretch is more likely to be reflective of short-term changes in parameters such as fluid balance and cardiac filling pressures than the longer-term adverse changes such as remodelling which are associated with myocyte hypertrophy. This could explain the differences found between the ability of changes

in the different biomarkers to predict CV admission over a longer time period.

Future studies may therefore focus on assessing if certain biomarkers can be linked with monitoring particular aetiological and phenotypic presentations within the vast cohort of patients labelled with a chronic HF diagnosis. Such individualised care remains the ultimate goal of modern medicine, but is likely to require approaches based on genetic factors as well as recognised pathophysiological processes and clinical parameters. Indeed, with prognosis intricately linked to all such factors, it is likely that the multi-marker approach, utilising biomarkers reflecting several different processes, will be the ultimate end-point for any monitoring pathway. Unfortunately, multi-marker analysis of the results in this thesis was not possible, but should certainly be a focus of future research in the field of biomarker monitoring.

In conclusion, this thesis has provided the foundation for future work on selecting novel biomarkers for monitoring patients with chronic HF. Results suggest that more focus on underlying pathological processes may be key in biomarker selection, with those reflecting myocyte stretch unlikely to provide long-term success in this area. Selection based on biological variability alone is not recommended. Galectin-3 and sST2 appear the most promising of the biomarkers studied. Further randomised controlled trials examining the effect of using serial changes in sST2 and galectin-3 to monitor patients in a larger HF cohort is recommended to validate these conclusions.

PUBLICATIONS ARISING FROM THIS THESIS

Original Articles

2016 The biological variability of sST2 in patients with chronic stable heart failure

Piper SE; deCourcey J, Sherwood RA, Amin-Youssef GF, McDonagh TA

Am J Cardiol. 2016 Jul 1;118(1):95-8

2016 Serial galectin-3 for the monitoring of optimally treated stable chronic heart failure: a pilot study

Piper SE, deCourcey J, Sherwood RA, Amin-Youssef GF, McDonagh TA

International Journal of Cardiology. 2016 Mar 15;207:279-81

2015 Serial soluble ST2 for the monitoring of pharmacologically optimised chronic stable heart failure

Piper SE, Sherwood RA, Amin-Youssef GF, Shah AM, McDonagh TA

International Journal of Cardiology. 2015 Jan 15;178:284-91

2014 Heart Failure: The role of natriuretic peptides in diagnosis

Piper SE.

British Journal of Cardiology E-Learning Programme on Heart Failure

<http://bjcardio.co.uk/2014/09/heart-failure-module-2-diagnosis/5/>

2011 Brain natriuretic peptide in chronic heart failure: An update.

Piper SE and McDonagh TA.

Cardiology News, Dec-Jan 2012

Abstracts

2016 The impact of biological variability on biomarker monitoring

Piper SE, deCoursey J, Sherwood R, Amin-Youssef G, McDonagh TA

Poster Presentation. Heart Failure Association of the ESC Congress, Florence, Italy

2015 Changes in novel biomarkers of hypertrophy and fibrosis are better predictors of CV admission than those of cardiac myocyte stretch in patients with CHF

Piper SE, Hipperson D, deCoursey J, Sherwood R, Amin-Youssef G, Shah AJ, McDonagh TA

Poster Presentation. Heart Failure Association of the ESC Congress, Seville, Spain

2014 Novel biomarkers to predict cardiovascular admission in patients with pharmacologically optimized chronic heart failure

Piper SE, Hipperson D, deCoursey J, Sherwood R, Amin-Youssef G, Shah AJ, McDonagh TA

Rapid Fire Abstract Presentation. ESC Congress, Barcelona, Spain.

2014 The biological variability of sST2 in chronic heart failure

Piper SE, Hipperson D, deCoursey J, Sherwood R, Amin-Youssef G, Shah AJ, McDonagh TA

Poster Presentation. British Cardiovascular Society Conference, Manchester, UK

2014 The biological variability of galectin-3 in chronic heart failure.

Piper SE, Hipperson D, deCoursey J, Sherwood R, Amin-Youssef G, Shah AJ, McDonagh TA

Poster Presentation. Heart Failure Association of the ESC Congress, Athens, Greece

2014 The biological variability of mid-regional pro-adrenomedullin in chronic heart failure

Piper SE, Hipperson D, deCoursey J, Sherwood R, Amin-Youssef G, Shah AJ,
McDonagh TA

Poster Presentation. Heart Failure Association of the ESC Congress, Athens, Greece

2014 Serial galectin-3 measurements predict cardiovascular admission better than NTproBNP

Piper SE, Hipperson D, deCoursey J, Sherwood R, Amin-Youssef G, McDonagh TA

Poster Presentation. Heart Failure Association of the ESC Congress, Athens, Greece

2013 Monitoring of chronic heart failure with serum soluble ST2.

Piper SE, Hipperson D, deCoursey J, Sherwood R, Amin-Youssef G, Shah AJ,
McDonagh TA

Poster Presentation. Heart Failure Association of the ESC Congress, Lisbon, Portugal

OTHER PUBLICATIONS

Review Articles

2015 Chemotherapy related cardiomyopathy

Piper SE and McDonagh TA

European Cardiology Review. 2015 July;10(1):19-24

2015 Heart failure and chemotherapeutic agents: A review

Piper SE and McDonagh TA

Future Cardiology (2015) 11(4), 453–470

2014 The role of intravenous vasodilators in acute heart failure management

Piper SE and McDonagh TA

European Journal of Heart Failure. 2014 July;16:827-834.

Abstracts

2016 Novel biomarkers in assessing outcome in patients with severe aortic stenosis and heart failure

Cai A, Dworakowski R, Sunderland N, Piper SE, Jegard N, Gibbs T, Redding S, Wendler O, Byrne J, MacCarthy P, McDonagh TA

Poster Presentation. British Cardiovascular Society Conference, Manchester

2015 Acute heart failure: where did we go wrong?

Shoaib A, Piper S, John J, Oliver R, Wong K, Rigby A, Hardman S, Clark AL, McDonagh T, Cleland JG

Poster Presentation. British Cardiovascular Society Conference, Manchester

- 2015 Reasons for admitting a patient for heart failure: where did we go wrong?**
- Shoaib A, Piper S, John J, Oliver R, Wong K, Rigby A, Hardman S, Clark AL, McDonagh T, Cleland JG
- Moderated Poster.** Heart Failure Association of the ESC Congress, Seville, Spain.
-
- 2014 Acute heart failure patients – a deep dive into the UK National Heart Failure Audit**
- Piper SE, Shoaib A, Mitchell P, Dargie HJ, Cleland JC, Hardman SE, McDonagh T A.
- Poster Presentation. Heart Failure Association of the ESC Congress, Athens, Greece
-
- 2013 Myocardial scar on cardiovascular magnetic resonance Imaging predicts adverse outcome in adults with non-Ischaemic dilated cardiomyopathy**
- Plymen CM, Piper SE, Hilton J, Rajani R, Carr-White GS, Kapetanakis S.
- Poster Presentation. Heart Failure Association of the ESC Congress, Lisbon, Portugal
-
- 2013 Treating iron deficiency in heart failure evaluation (TIDE-HF)**
- Hipperson D, Piper SE, Plymen CM, Breeze J, deCoursey J, Amin-Youssef G, McDonagh TA.
- Poster Presentation. Heart Failure Society of the ESC Congress, Lisbon, Portugal

REFERENCES

1. Huckabee W, Casten G, Harrison TR. Experimental hypervolemic heart failure; its bearing on certain general principles of heart failure. *Circulation*. 1950;1(3):343-56.
2. Kranias Lab E. Kranias Lab : Heart Failure 2015 [Available from: http://med2.uc.edu/kranias/heart_failure.htm].
3. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). The CONSENSUS Trial Study Group. *The New England journal of medicine*. 1987;316(23):1429-35.
4. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. The SOLVD Investigators. *The New England journal of medicine*. 1991;325(5):293-302.
5. The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. *Lancet*. 1999;353(9146):9-13.
6. Dargie HJ. Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. *Lancet*. 2001;357(9266):1385-90.
7. McMurray JJ, Pfeffer MA. Heart failure. *Lancet*. 2005;365(9474):1877-89.
8. Zannad F, McMurray JJ, Drexler H, Krum H, van Veldhuisen DJ, Swedberg K, et al. Rationale and design of the Eplerenone in Mild Patients Hospitalization And Survival Study in Heart Failure (EMPHASIS-HF). *Eur J Heart Fail*. 2010;12(6):617-22.
9. McDonagh TA, Morrison CE, Lawrence A, Ford I, Tunstall-Pedoe H, McMurray JJ, et al. Symptomatic and asymptomatic left-ventricular systolic dysfunction in an urban population. *Lancet*. 1997;350(9081):829-33.
10. Gardin JM, Siscovick D, Anton-Culver H, Lynch JC, Smith VE, Klopfenstein HS, et al. Sex, age, and disease affect echocardiographic left ventricular mass and systolic function in the free-living elderly. The Cardiovascular Health Study. *Circulation*. 1995;91(6):1739-48.

11. Hedberg P, Lonnberg I, Jonason T, Nilsson G, Pehrsson K, Ringqvist I. Left ventricular systolic dysfunction in 75-year-old men and women; a population-based study. *Eur Heart J*. 2001;22(8):676-83.
12. Ceia F, Fonseca C, Mota T, Morais H, Matias F, de Sousa A, et al. Prevalence of chronic heart failure in Southwestern Europe: the EPICA study. *Eur J Heart Fail*. 2002;4(4):531-9.
13. Cortina A, Reguero J, Segovia E, Rodriguez Lambert JL, Cortina R, Arias JC, et al. Prevalence of heart failure in Asturias (a region in the north of Spain). *Am J Cardiol*. 2001;87(12):1417-9.
14. Mosterd A, Hoes AW, de Bruyne MC, Deckers JW, Linker DT, Hofman A, et al. Prevalence of heart failure and left ventricular dysfunction in the general population; The Rotterdam Study. *Eur Heart J*. 1999;20(6):447-55.
15. Nielsen OW, Hilden J, Larsen CT, Hansen JF. Cross sectional study estimating prevalence of heart failure and left ventricular systolic dysfunction in community patients at risk. *Heart*. 2001;86(2):172-8.
16. Redfield MM, Jacobsen SJ, Burnett JC, Jr., Mahoney DW, Bailey KR, Rodeheffer RJ. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *Jama*. 2003;289(2):194-202.
17. Ponikowski PA, S. Alhabib, K. Cowie, M. Force, T. Hu, S. Jaarsma, T. Krum, H. Rastogi, V. Rohde, L. Samal, U. Shimokawa, H. Siswanto, BB. Silwa K. Filippatos G. Heart Failure: Preventing Disease and Death Worldwide. 2014.
18. Davies M, Hobbs F, Davis R, Kenkre J, Roalfe AK, Hare R, et al. Prevalence of left-ventricular systolic dysfunction and heart failure in the Echocardiographic Heart of England Screening study: a population based study. *Lancet*. 2001;358(9280):439-44.
19. Cowie MR, Wood DA, Coats AJ, Thompson SG, Poole-Wilson PA, Suresh V, et al. Incidence and aetiology of heart failure; a population-based study. *Eur Heart J*. 1999;20(6):421-8.
20. Research NifCO. National Heart Failure Audit 2014/2015. 2015.
21. NICE. CG108 Chronic heart failure: NICE guideline. 2010.

22. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Bohm M, Dickstein K, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail.* 2012;14(8):803-69.
23. RE K. Cardiovascular physiology concepts. 2nd ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2012.
24. Mohrman DE HL. Cardiovascular physiology. New York: McGraw-Hill; 2010.
25. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, et al. Myocardial infarct size and ventricular function in rats. *Circulation research.* 1979;44(4):503-12.
26. Spadaro J, Fishbein MC, Hare C, Pfeffer MA, Maroko PR. Characterization of myocardial infarcts in the rat. *Archives of pathology & laboratory medicine.* 1980;104(4):179-83.
27. Konstam MA, Udelson JE, Anand IS, Cohn JN. Ventricular remodeling in heart failure: a credible surrogate endpoint. *J Card Fail.* 2003;9(5):350-3.
28. Hwang JJ, Dzau VJ, Liew CC. Genomics and the pathophysiology of heart failure. *Current cardiology reports.* 2001;3(3):198-207.
29. Jackson G, Gibbs CR, Davies MK, Lip GYH. ABC of Heart Failure: Pathophysiology. *British medical journal.* 2000;320:167-70.
30. Brilla CG, Maisch B. Regulation of the structural remodelling of the myocardium: from hypertrophy to heart failure. *Eur Heart J.* 1994;15 Suppl D:45-52.
31. Remes J, Miettinen H, Reunanen A, Pyorala K. Validity of clinical diagnosis of heart failure in primary health care. *Eur Heart J.* 1991;12(3):315-21.
32. Cleland JG, Swedberg K, Follath F, Komajda M, Cohen-Solal A, Aguilar JC, et al. The EuroHeart Failure survey programme-- a survey on the quality of care among patients with heart failure in Europe. Part 1: patient characteristics and diagnosis. *Eur Heart J.* 2003;24(5):442-63.

33. Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, et al. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary. *The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation*. 2002;21(2):189-203.
34. Nieminen MS, Bohm M, Cowie MR, Drexler H, Filippatos GS, Jondeau G, et al. Executive summary of the guidelines on the diagnosis and treatment of acute heart failure: the Task Force on Acute Heart Failure of the European Society of Cardiology. *Eur Heart J*. 2005;26(4):384-416.
35. Swedberg K, Cleland J, Dargie H, Drexler H, Follath F, Komajda M, et al. [Guidelines for the Diagnosis and Treatment of Chronic Heart Failure: executive summary (update 2005)]. *Revista espanola de cardiologia*. 2005;58(9):1062-92.
36. ACC/AHA collaborate on guidelines for the evaluation and management of heart failure. *American family physician*. 1996;53(6):2196-8.
37. Ruschitzka F, Abraham WT, Singh JP, Bax JJ, Borer JS, Brugada J, et al. Cardiac-resynchronization therapy in heart failure with a narrow QRS complex. *The New England journal of medicine*. 2013;369(15):1395-405.
38. Garg R, Yusuf S. Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. Collaborative Group on ACE Inhibitor Trials. *Jama*. 1995;273(18):1450-6.
39. Packer M, Poole-Wilson PA, Armstrong PW, Cleland JG, Horowitz JD, Massie BM, et al. Comparative effects of low and high doses of the angiotensin-converting enzyme inhibitor, lisinopril, on morbidity and mortality in chronic heart failure. ATLAS Study Group. *Circulation*. 1999;100(23):2312-8.
40. Flather MD, Yusuf S, Kober L, Pfeffer M, Hall A, Murray G, et al. Long-term ACE-inhibitor therapy in patients with heart failure or left-ventricular dysfunction: a systematic overview of data from individual patients. ACE-Inhibitor Myocardial Infarction Collaborative Group. *Lancet*. 2000;355(9215):1575-81.

41. Kiowski W, Sutsch G, Dossegger L. Clinical benefit of angiotensin-converting enzyme inhibitors in chronic heart failure. *Journal of cardiovascular pharmacology*. 1996;27 Suppl 2:S19-24.
42. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ, Jr., Cuddy TE, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. *The New England journal of medicine*. 1992;327(10):669-77.
43. Forker AD, Wilson CS. Congestive heart failure. A new iatrogenic epidemic, propranolol (Inderal) induced. *The Journal of the Kansas Medical Society*. 1974;75(2):33-6.
44. Julian DG. Can beta blockers be safely used in patients with recent myocardial infarction who also have congestive heart failure? *Circulation*. 1983;67(6 Pt 2):I91.
45. Strauer BE. Beta-blocking agents in heart failure: modern concepts and overview. *Journal of cardiovascular pharmacology*. 1990;16 Suppl 5:S129-32.
46. Packer M, Fowler MB, Roecker EB, Coats AJ, Katus HA, Krum H, et al. Effect of carvedilol on the morbidity of patients with severe chronic heart failure: results of the carvedilol prospective randomized cumulative survival (COPERNICUS) study. *Circulation*. 2002;106(17):2194-9.
47. Hjalmarson A, Goldstein S, Fagerberg B, Wedel H, Waagstein F, Kjeksbus J, et al. Effects of controlled-release metoprolol on total mortality, hospitalizations, and well-being in patients with heart failure: the Metoprolol CR/XL Randomized Intervention Trial in congestive heart failure (MERIT-HF). MERIT-HF Study Group. *Jama*. 2000;283(10):1295-302.
48. Flather MD, Shibata MC, Coats AJ, Van Veldhuisen DJ, Parkhomenko A, Borbola J, et al. Randomized trial to determine the effect of nebivolol on mortality and cardiovascular hospital admission in elderly patients with heart failure (SENIORS). *Eur Heart J*. 2005;26(3):215-25.
49. Heidenreich PA, Lee TT, Massie BM. Effect of beta-blockade on mortality in patients with heart failure: a meta-analysis of randomized clinical trials. *J Am Coll Cardiol*. 1997;30(1):27-34.

50. Lechat P, Packer M, Chalon S, Cucherat M, Arab T, Boissel JP. Clinical effects of beta-adrenergic blockade in chronic heart failure: a meta-analysis of double-blind, placebo-controlled, randomized trials. *Circulation*. 1998;98(12):1184-91.
51. Packer M. Effects of beta-adrenergic blockade on survival of patients with chronic heart failure. *Am J Cardiol*. 1997;80(11a):46I-54I.
52. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM, et al. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. U.S. Carvedilol Heart Failure Study Group. *The New England journal of medicine*. 1996;334(21):1349-55.
53. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *The New England journal of medicine*. 1999;341(10):709-17.
54. Zannad F, McMurray JJ, Krum H, van Veldhuisen DJ, Swedberg K, Shi H, et al. Eplerenone in patients with systolic heart failure and mild symptoms. *The New England journal of medicine*. 2011;364(1):11-21.
55. Granger CB, McMurray JJ, Yusuf S, Held P, Michelson EL, Olofsson B, et al. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. *Lancet*. 2003;362(9386):772-6.
56. The effect of digoxin on mortality and morbidity in patients with heart failure. *The New England journal of medicine*. 1997;336(8):525-33.
57. Bristow MR, Gilbert EM, Abraham WT, Adams KF, Fowler MB, Hershberger RE, et al. Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. MOCHA Investigators. *Circulation*. 1996;94(11):2807-16.

58. Swedberg K, Komajda M, Bohm M, Borer JS, Ford I, Dubost-Brama A, et al. Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study. *Lancet*. 2010;376(9744):875-85.
59. Ivabradine for treating chronic heart failure | 1-guidance | Guidance and guidelines | NICE. NICE; 2014.
60. Cohn JN, Archibald DG, Ziesche S, Franciosa JA, Harston WE, Tristani FE, et al. Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a Veterans Administration Cooperative Study. *The New England journal of medicine*. 1986;314(24):1547-52.
61. Cohn JN, Johnson G, Ziesche S, Cobb F, Francis G, Tristani F, et al. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. *The New England journal of medicine*. 1991;325(5):303-10.
62. Carson P, Ziesche S, Johnson G, Cohn JN. Racial differences in response to therapy for heart failure: analysis of the vasodilator-heart failure trials. *Vasodilator-Heart Failure Trial Study Group. J Card Fail*. 1999;5(3):178-87.
63. Taylor AL, Ziesche S, Yancy C, Carson P, D'Agostino R, Jr., Ferdinand K, et al. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. *The New England journal of medicine*. 2004;351(20):2049-57.
64. McMurray JJ, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *The New England journal of medicine*. 2014;371(11):993-1004.
65. Kuhn M. Molecular physiology of natriuretic peptide signalling. *Basic research in cardiology*. 2004;99(2):76-82.
66. Bardy GH, Lee KL, Mark DB, Poole JE, Packer DL, Boineau R, et al. Amiodarone or an implantable cardioverter-defibrillator for congestive heart failure. *The New England journal of medicine*. 2005;352(3):225-37.

67. Kadish A, Dyer A, Daubert JP, Quigg R, Estes NA, Anderson KP, et al. Prophylactic defibrillator implantation in patients with nonischemic dilated cardiomyopathy. *The New England journal of medicine*. 2004;350(21):2151-8.
68. Moss AJ, Hall WJ, Cannom DS, Daubert JP, Higgins SL, Klein H, et al. Improved survival with an implanted defibrillator in patients with coronary disease at high risk for ventricular arrhythmia. Multicenter Automatic Defibrillator Implantation Trial Investigators. *The New England journal of medicine*. 1996;335(26):1933-40.
69. Bristow MR, Saxon LA, Boehmer J, Krueger S, Kass DA, De Marco T, et al. Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. *The New England journal of medicine*. 2004;350(21):2140-50.
70. Cleland JG, Daubert JC, Erdmann E, Freemantle N, Gras D, Kappenberger L, et al. The effect of cardiac resynchronization on morbidity and mortality in heart failure. *The New England journal of medicine*. 2005;352(15):1539-49.
71. Moss AJ, Hall WJ, Cannom DS, Klein H, Brown MW, Daubert JP, et al. Cardiac-resynchronization therapy for the prevention of heart-failure events. *The New England journal of medicine*. 2009;361(14):1329-38.
72. Tang AS, Wells GA, Talajic M, Arnold MO, Sheldon R, Connolly S, et al. Cardiac-resynchronization therapy for mild-to-moderate heart failure. *The New England journal of medicine*. 2010;363(25):2385-95.
73. Linde C, Abraham WT, Gold MR, St John Sutton M, Ghio S, Daubert C. Randomized trial of cardiac resynchronization in mildly symptomatic heart failure patients and in asymptomatic patients with left ventricular dysfunction and previous heart failure symptoms. *J Am Coll Cardiol*. 2008;52(23):1834-43.
74. Leclercq C, Walker S, Linde C, Clementy J, Marshall AJ, Ritter P, et al. Comparative effects of permanent biventricular and right-univentricular pacing in heart failure patients with chronic atrial fibrillation. *Eur Heart J*. 2002;23(22):1780-7.

75. Wilton SB, Leung AA, Ghali WA, Faris P, Exner DV. Outcomes of cardiac resynchronization therapy in patients with versus those without atrial fibrillation: a systematic review and meta-analysis. *Heart rhythm : the official journal of the Heart Rhythm Society*. 2011;8(7):1088-94.
76. Upadhyay GA, Choudhry NK, Auricchio A, Ruskin J, Singh JP. Cardiac resynchronization in patients with atrial fibrillation: a meta-analysis of prospective cohort studies. *J Am Coll Cardiol*. 2008;52(15):1239-46.
77. Gasparini M, Leclercq C, Lunati M, Landolina M, Auricchio A, Santini M, et al. Cardiac resynchronization therapy in patients with atrial fibrillation: the CERTIFY study (Cardiac Resynchronization Therapy in Atrial Fibrillation Patients Multinational Registry). *JACC Heart failure*. 2013;1(6):500-7.
78. Thiele H, Zeymer U, Neumann FJ, Ferenc M, Olbrich HG, Hausleiter J, et al. Intraaortic balloon support for myocardial infarction with cardiogenic shock. *The New England journal of medicine*. 2012;367(14):1287-96.
79. Thiele H, Zeymer U, Neumann FJ, Ferenc M, Olbrich HG, Hausleiter J, et al. Intra-aortic balloon counterpulsation in acute myocardial infarction complicated by cardiogenic shock (IABP-SHOCK II): final 12 month results of a randomised, open-label trial. *Lancet*. 2013;382(9905):1638-45.
80. Zeymer U, Hochadel M, Hauptmann KE, Wiegand K, Schuhmacher B, Brachmann J, et al. Intra-aortic balloon pump in patients with acute myocardial infarction complicated by cardiogenic shock: results of the ALKK-PCI registry. *Clinical research in cardiology : official journal of the German Cardiac Society*. 2013;102(3):223-7.
81. Basra SS, Loyalka P, Kar B. Current status of percutaneous ventricular assist devices for cardiogenic shock. *Current opinion in cardiology*. 2011;26(6):548-54.
82. Thiele H, Sick P, Boudriot E, Diederich KW, Hambrecht R, Niebauer J, et al. Randomized comparison of intra-aortic balloon support with a percutaneous left ventricular assist device in

patients with revascularized acute myocardial infarction complicated by cardiogenic shock. *Eur Heart J*. 2005;26(13):1276-83.

83. Prondzinsky R, Unverzagt S, Russ M, Lemm H, Swyter M, Wegener N, et al. Hemodynamic effects of intra-aortic balloon counterpulsation in patients with acute myocardial infarction complicated by cardiogenic shock: the prospective, randomized IABP shock trial. *Shock (Augusta, Ga)*. 2012;37(4):378-84.

84. Seyfarth M, Sibbing D, Bauer I, Frohlich G, Bott-Flugel L, Byrne R, et al. A randomized clinical trial to evaluate the safety and efficacy of a percutaneous left ventricular assist device versus intra-aortic balloon pumping for treatment of cardiogenic shock caused by myocardial infarction. *J Am Coll Cardiol*. 2008;52(19):1584-8.

85. Cheng JM, den Uil CA, Hoeks SE, van der Ent M, Jewbali LS, van Domburg RT, et al. Percutaneous left ventricular assist devices vs. intra-aortic balloon pump counterpulsation for treatment of cardiogenic shock: a meta-analysis of controlled trials. *Eur Heart J*. 2009;30(17):2102-8.

86. Lawson WE, Koo M. Percutaneous Ventricular Assist Devices and ECMO in the Management of Acute Decompensated Heart Failure. *Clin Med Insights Cardiol*. 2015;9(Suppl 1):41-8.

87. Basir MB, Schreiber TL, Grines CL, Dixon SR, Moses JW, Maini BS, et al. Effect of Early Initiation of Mechanical Circulatory Support on Survival in Cardiogenic Shock. *Am J Cardiol*. 2017;119(6):845-51.

88. Burkhoff D, Cohen H, Brunckhorst C, O'Neill WW. A randomized multicenter clinical study to evaluate the safety and efficacy of the TandemHeart percutaneous ventricular assist device versus conventional therapy with intraaortic balloon pumping for treatment of cardiogenic shock. *American heart journal*. 2006;152(3):469.e1-8.

89. Transplant NBa. NHSBT - ODT Clinical Site - Transplant Organ Specific Reports - Cardiac Transplant. 2015.

90. Trivedi JR, Cheng A, Singh R, Williams ML, Slaughter MS. Survival on the heart transplant waiting list: impact of continuous flow left ventricular assist device as bridge to transplant. *The Annals of thoracic surgery*. 2014;98(3):830-4.
91. Westaby S. Cardiac transplant or rotary blood pump: contemporary evidence. *The Journal of thoracic and cardiovascular surgery*. 2013;145(1):24-31.
92. Tozzi P, Hullin R. Mechanical circulatory support for destination therapy. *Swiss medical weekly*. 2016;146:w14267.
93. Transplant NBa. NHSBT - ODT Clinical Site - Transplant Organ Specific Reports - VAD. 2015.
94. Transplant NBa. NHSBT - ODT Clinical Site - Transplant Organ Specific Reports. 2014.
95. Clarke A, Pulikottil-Jacob R, Connock M, Suri G, Kandala NB, Maheswaran H, et al. Cost-effectiveness of left ventricular assist devices (LVADs) for patients with advanced heart failure: analysis of the British NHS bridge to transplant (BTT) program. *International journal of cardiology*. 2014;171(3):338-45.
96. Banner NR, Bonser RS, Clark AL, Clark S, Cowburn PJ, Gardner RS, et al. UK guidelines for referral and assessment of adults for heart transplantation. *Heart*. 2011;97(18):1520-7.
97. Hamour IM, Khaghani A, Kanagala PK, Mitchell AG, Banner NR. Current outcome of heart transplantation: a 10-year single centre perspective and review. *QJM : monthly journal of the Association of Physicians*. 2011;104(4):335-43.
98. Stewart S, MacIntyre K, Hole DJ, Capewell S, McMurray JJ. More 'malignant' than cancer? Five-year survival following a first admission for heart failure. *Eur J Heart Fail*. 2001;3(3):315-22.
99. Askoxylakis V, Thieke C, Pleger ST, Most P, Tanner J, Lindel K, et al. Long-term survival of cancer patients compared to heart failure and stroke: a systematic review. *BMC cancer*. 2010;10:105.
100. Cowie MR, Wood DA, Coats AJ, Thompson SG, Suresh V, Poole-Wilson PA, et al. Survival of patients with a new diagnosis of heart failure: a population based study. *Heart*. 2000;83(5):505-10.

101. Cleland JG, McDonagh T, Rigby AS, Yassin A, Whittaker T, Dargie HJ. The national heart failure audit for England and Wales 2008-2009. *Heart*. 2011;97(11):876-86.
102. NICOR. Heart Failure Audit 2010-2011. 2011.
103. Cleland J DH, Hardman S, McDonagh T, Mitchell P. National Heart Failure Audit: April 2011-March 2012. London: National Institute for Cardiovascular Outcomes Research; 2012.
104. Sliwa K, Davison BA, Mayosi BM, Damasceno A, Sani M, Ogah OS, et al. Readmission and death after an acute heart failure event: predictors and outcomes in sub-Saharan Africa: results from the THESUS-HF registry. *Eur Heart J*. 2013;34(40):3151-9.
105. Wasywich CA, Gamble GD, Whalley GA, Doughty RN. Understanding changing patterns of survival and hospitalization for heart failure over two decades in New Zealand: utility of 'days alive and out of hospital' from epidemiological data. *Eur J Heart Fail*. 2010;12(5):462-8.
106. Fonarow GC, Heywood JT, Heidenreich PA, Lopatin M, Yancy CW. Temporal trends in clinical characteristics, treatments, and outcomes for heart failure hospitalizations, 2002 to 2004: findings from Acute Decompensated Heart Failure National Registry (ADHERE). *American heart journal*. 2007;153(6):1021-8.
107. Klersy C, Boriani G, De Silvestri A, Mairesse GH, Braunschweig F, Scotti V, et al. Effect of telemonitoring of cardiac implantable electronic devices on healthcare utilization: a meta-analysis of randomized controlled trials in patients with heart failure. *Eur J Heart Fail*. 2016;18(2):195-204.
108. Klersy C, De Silvestri A, Gabutti G, Regoli F, Auricchio A. A meta-analysis of remote monitoring of heart failure patients. *J Am Coll Cardiol*. 2009;54(18):1683-94.
109. Pandor A, Gomersall T, Stevens JW, Wang J, Al-Mohammad A, Bakhai A, et al. Remote monitoring after recent hospital discharge in patients with heart failure: a systematic review and network meta-analysis. *Heart*. 2013;99(23):1717-26.
110. Abraham WT, Compton S, Haas G, Foreman B, Canby RC, Fishel R, et al. Intrathoracic impedance vs daily weight monitoring for predicting worsening heart failure events: results of the

Fluid Accumulation Status Trial (FAST). *Congestive heart failure* (Greenwich, Conn). 2011;17(2):51-5.

111. van Veldhuisen DJ, Braunschweig F, Conraads V, Ford I, Cowie MR, Jondeau G, et al. Intrathoracic impedance monitoring, audible patient alerts, and outcome in patients with heart failure. *Circulation*. 2011;124(16):1719-26.

112. Ypenburg C, Bax JJ, van der Wall EE, Schalij MJ, van Erven L. Intrathoracic impedance monitoring to predict decompensated heart failure. *Am J Cardiol*. 2007;99(4):554-7.

113. Adamson PB, Smith AL, Abraham WT, Kleckner KJ, Stadler RW, Shih A, et al. Continuous autonomic assessment in patients with symptomatic heart failure: prognostic value of heart rate variability measured by an implanted cardiac resynchronization device. *Circulation*. 2004;110(16):2389-94.

114. Bourge RC, Abraham WT, Adamson PB, Aaron MF, Aranda JM, Jr., Magalski A, et al. Randomized controlled trial of an implantable continuous hemodynamic monitor in patients with advanced heart failure: the COMPASS-HF study. *J Am Coll Cardiol*. 2008;51(11):1073-9.

115. Ritzema J, Troughton R, Melton I, Crozier I, Doughty R, Krum H, et al. Physician-directed patient self-management of left atrial pressure in advanced chronic heart failure. *Circulation*. 2010;121(9):1086-95.

116. Whellan DJ, Ousdigian KT, Al-Khatib SM, Pu W, Sarkar S, Porter CB, et al. Combined heart failure device diagnostics identify patients at higher risk of subsequent heart failure hospitalizations: results from PARTNERS HF (Program to Access and Review Trending Information and Evaluate Correlation to Symptoms in Patients With Heart Failure) study. *J Am Coll Cardiol*. 2010;55(17):1803-10.

117. Abraham WT, Adamson PB, Bourge RC, Aaron MF, Costanzo MR, Stevenson LW, et al. Wireless pulmonary artery haemodynamic monitoring in chronic heart failure: a randomised controlled trial. *Lancet*. 2011;377(9766):658-66.

118. Abraham WT, Stevenson LW, Bourge RC, Lindenfeld JA, Bauman JG, Adamson PB. Sustained efficacy of pulmonary artery pressure to guide adjustment of chronic heart failure therapy: complete follow-up results from the CHAMPION randomised trial. *Lancet*. 2016;387(10017):453-61.
119. Hindricks G, Taborsky M, Glikson M, Heinrich U, Schumacher B, Katz A, et al. Implant-based multiparameter telemonitoring of patients with heart failure (IN-TIME): a randomised controlled trial. *Lancet*. 2014;384(9943):583-90.
120. Chaudhry SI, Mattera JA, Curtis JP, Spertus JA, Herrin J, Lin Z, et al. Telemonitoring in patients with heart failure. *The New England journal of medicine*. 2010;363(24):2301-9.
121. Koehler F, Winkler S, Schieber M, Sechtem U, Stangl K, Bohm M, et al. Impact of remote telemedical management on mortality and hospitalizations in ambulatory patients with chronic heart failure: the telemedical interventional monitoring in heart failure study. *Circulation*. 2011;123(17):1873-80.
122. Ferrante D, Varini S, Macchia A, Soifer S, Badra R, Nul D, et al. Long-term results after a telephone intervention in chronic heart failure: DIAL (Randomized Trial of Phone Intervention in Chronic Heart Failure) follow-up. *J Am Coll Cardiol*. 2010;56(5):372-8.
123. Krum H, Forbes A, Yallop J, Driscoll A, Croucher J, Chan B, et al. Telephone support to rural and remote patients with heart failure: the Chronic Heart Failure Assessment by Telephone (CHAT) study. *Cardiovascular therapeutics*. 2013;31(4):230-7.
124. Ramachandran K, Husain N, Maikhuri R, Seth S, Vij A, Kumar M, et al. Impact of a comprehensive telephone-based disease management programme on quality-of-life in patients with heart failure. *The National medical journal of India*. 2007;20(2):67-73.
125. Riegel B, Carlson B, Kopp Z, LePetri B, Glaser D, Unger A. Effect of a standardized nurse case-management telephone intervention on resource use in patients with chronic heart failure. *Archives of internal medicine*. 2002;162(6):705-12.

126. Wakefield BJ, Ward MM, Holman JE, Ray A, Scherubel M, Burns TL, et al. Evaluation of home telehealth following hospitalization for heart failure: a randomized trial. *Telemedicine journal and e-health : the official journal of the American Telemedicine Association*. 2008;14(8):753-61.
127. Anker SD, Koehler F, Abraham WT. Telemedicine and remote management of patients with heart failure. *Lancet*. 2011;378(9792):731-9.
128. Pandor A, Thokala P, Gomersall T, Baalbaki H, Stevens JW, Wang J, et al. Home telemonitoring or structured telephone support programmes after recent discharge in patients with heart failure: systematic review and economic evaluation. *Health technology assessment (Winchester, England)*. 2013;17(32):1-207, v-vi.
129. Berger R, Moertl D, Peter S, Ahmadi R, Huelsmann M, Yamuti S, et al. N-terminal pro-B-type natriuretic peptide-guided, intensive patient management in addition to multidisciplinary care in chronic heart failure a 3-arm, prospective, randomized pilot study. *J Am Coll Cardiol*. 2010;55(7):645-53.
130. Eurlings LW, van Pol PE, Kok WE, van Wijk S, Lodewijks-van der Bolt C, Balk AH, et al. Management of chronic heart failure guided by individual N-terminal pro-B-type natriuretic peptide targets: results of the PRIMA (Can PRO-brain-natriuretic peptide guided therapy of chronic heart failure IMprove heart fAilure morbidity and mortality?) study. *J Am Coll Cardiol*. 2010;56(25):2090-100.
131. Gardner RS, Chong KS, Morton JJ, McDonagh TA. A change in N-terminal pro-brain natriuretic peptide is predictive of outcome in patients with advanced heart failure. *Eur J Heart Fail*. 2007;9(3):266-71.
132. Januzzi JL, Rehman SU, Mohammed AA, Bhardwaj A, Barajas L, Barajas J, et al. Use of amino-terminal pro-B-type natriuretic peptide to guide outpatient therapy of patients with chronic left ventricular systolic dysfunction. *J Am Coll Cardiol*. 2011;58(18):1881-9.

133. Jourdain P, Jondeau G, Funck F, Gueffet P, Le Helloco A, Donal E, et al. Plasma brain natriuretic peptide-guided therapy to improve outcome in heart failure: the STARS-BNP Multicenter Study. *J Am Coll Cardiol*. 2007;49(16):1733-9.
134. McDonagh TA, Gardner RS, Chong KS, Dargie HJ. Can we use B-type natriuretic peptides to monitor patients with heart failure? *Biomarkers in medicine*. 2007;1(3):349-53.
135. Shah MR, Califf RM, Nohria A, Bhapkar M, Bowers M, Mancini DM, et al. The STARBRITE trial: a randomized, pilot study of B-type natriuretic peptide-guided therapy in patients with advanced heart failure. *J Card Fail*. 2011;17(8):613-21.
136. Motiwala SR, Szymonifka J, Belcher A, Weiner RB, Baggish AL, Sluss P, et al. Serial measurement of galectin-3 in patients with chronic heart failure: results from the ProBNP Outpatient Tailored Chronic Heart Failure Therapy (PROTECT) study. *Eur J Heart Fail*. 2013;15(10):1157-63.
137. van der Velde AR, Gullestad L, Ueland T, Aukrust P, Guo Y, Adourian A, et al. Prognostic Value of Changes in Galectin-3 Levels Over Time in Patients With Heart Failure: Data From CORONA and COACH. *Circ Heart Fail*. 2013;6(2):219-26.
138. Gaggin HK, Szymonifka J, Bhardwaj A, Belcher A, De Berardinis B, Motiwala S, et al. Head-to-head comparison of serial soluble ST2, growth differentiation factor-15, and highly-sensitive troponin T measurements in patients with chronic heart failure. *JACC Heart failure*. 2014;2(1):65-72.
139. BS H. Overview of biological markers. Hulka BS GJ, Wilcosky TC, editor. New York: Oxford University Press; 1990.
140. Strimbu K, Tavel JA. What are Biomarkers? *Curr Opin HIV AIDS*. 2010;5(6):463-6.
141. Mayeux R. Biomarkers: potential uses and limitations. *NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics*. 2004;1(2):182-8.
142. Pletcher MJ, Pignone M. Evaluating the clinical utility of a biomarker: a review of methods for estimating health impact. *Circulation*. 2011;123(10):1116-24.

143. Wilson JMG JG. Principles and practice of screening for disease. Geneva: World Health Organisation; 1968. p. 473.
144. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation*. 2006;113(19):2335-62.
145. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. *Critical reviews in clinical laboratory sciences*. 1989;27(5):409-37.
146. Petersen PH, Fraser CG, Sandberg S, Goldschmidt H. The index of individuality is often a misinterpreted quantity characteristic. *Clinical chemistry and laboratory medicine : CCLM / FESCC*. 1999;37(6):655-61.
147. Gaggin HK, Januzzi JL, Jr. Biomarkers and diagnostics in heart failure. *Biochim Biophys Acta*. 2013.
148. Kisch B. Electron microscopy of the atrium of the heart. I. Guinea pig. *Experimental medicine and surgery*. 1956;14(2-3):99-112.
149. Kangawa K, Fukuda A, Minamino N, Matsuo H. Purification and complete amino acid sequence of beta-rat atrial natriuretic polypeptide (beta-rANP) of 5,000 daltons. *Biochem Biophys Res Commun*. 1984;119(3):933-40.
150. Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, et al. Brain natriuretic peptide as a novel cardiac hormone in humans. Evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *The Journal of clinical investigation*. 1991;87(4):1402-12.
151. Hoffman A, Grossman E, Keiser HR. Increased plasma levels and blunted effects of brain natriuretic peptide in rats with congestive heart failure. *American journal of hypertension*. 1991;4(7 Pt 1):597-601.
152. Yoshimura M, Yasue H, Morita E, Sakaino N, Jougasaki M, Kurose M, et al. Hemodynamic, renal, and hormonal responses to brain natriuretic peptide infusion in patients with congestive heart failure. *Circulation*. 1991;84(4):1581-8.

153. Lang CC, Choy AM, Turner K, Tobin R, Coutie W, Struthers AD. The effect of intravenous saline loading on plasma levels of brain natriuretic peptide in man. *Journal of hypertension*. 1993;11(7):737-41.
154. Magga J, Marttila M, Mantymaa P, Vuolteenaho O, Ruskoaho H. Brain natriuretic peptide in plasma, atria, and ventricles of vasopressin- and phenylephrine-infused conscious rats. *Endocrinology*. 1994;134(6):2505-15.
155. Hall C. Essential biochemistry and physiology of (NT-pro)BNP. *Eur J Heart Fail*. 2004;6(3):257-60.
156. Piper SM, TA. Brain Natriuretic Peptide in Chronic Heart Failure: An Update. *Cardiology News*. 2012;15(2):6-11.
157. Mantymaa P, Vuolteenaho O, Marttila M, Ruskoaho H. Atrial stretch induces rapid increase in brain natriuretic peptide but not in atrial natriuretic peptide gene expression in vitro. *Endocrinology*. 1993;133(3):1470-3.
158. Kohno M, Horio T, Yasunari K, Yokokawa K, Ikeda M, Kurihara N, et al. Stimulation of brain natriuretic peptide release from the heart by thyroid hormone. *Metabolism: clinical and experimental*. 1993;42(8):1059-64.
159. Licata A, Corrao S, Petta S, Genco C, Cardillo M, Calvaruso V, et al. NT pro BNP plasma level and atrial volume are linked to the severity of liver cirrhosis. *PloS one*. 2013;8(8):e68364.
160. Yardan T, Altintop L, Baydin A, Yilmaz O, Guven H. B-type natriuretic peptide as an indicator of right ventricular dysfunction in acute pulmonary embolism. *International journal of clinical practice*. 2008;62(8):1177-82.
161. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC, Jr. Plasma brain natriuretic peptide concentration: impact of age and gender. *J Am Coll Cardiol*. 2002;40(5):976-82.

162. Beleigoli A, Diniz M, Nunes M, Barbosa M, Fernandes S, Abreu M, et al. Reduced brain natriuretic peptide levels in class III obesity: the role of metabolic and cardiovascular factors. *Obesity facts*. 2011;4(6):427-32.
163. Zaphiriou A, Robb S, Murray-Thomas T, Mendez G, Fox K, McDonagh T, et al. The diagnostic accuracy of plasma BNP and NTproBNP in patients referred from primary care with suspected heart failure: results of the UK natriuretic peptide study. *Eur J Heart Fail*. 2005;7(4):537-41.
164. Kelder JC, Cowie MR, McDonagh TA, Hardman SM, Grobbee DE, Cost B, et al. Quantifying the added value of BNP in suspected heart failure in general practice: an individual patient data meta-analysis. *Heart*. 2011;97(12):959-63.
165. Cowie MR, Struthers AD, Wood DA, Coats AJ, Thompson SG, Poole-Wilson PA, et al. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. *Lancet*. 1997;350(9088):1349-53.
166. McCullough PA, Nowak RM, McCord J, Hollander JE, Herrmann HC, Steg PG, et al. B-type natriuretic peptide and clinical judgment in emergency diagnosis of heart failure: analysis from Breathing Not Properly (BNP) Multinational Study. *Circulation*. 2002;106(4):416-22.
167. McDonagh TA, Robb SD, Murdoch DR, Morton JJ, Ford I, Morrison CE, et al. Biochemical detection of left-ventricular systolic dysfunction. *Lancet*. 1998;351(9095):9-13.
168. Maisel A, Krishnaswamy P, Nowak RM, McCord J, Hollander JE, Duc P, Omland T, Storrow AB, Abraham WT, Wu AHB, Clopton P, Steg PG, Westheim A, Wold Knudsen C, Perez A, Kazanegra R, Herrmann HC, McCullough PA. RAPID MEASUREMENT OF B-TYPE NATRIURETIC PEPTIDE. *New England Journal of Medicine*. 2002;347(3):161-7.
169. Januzzi JL, Jr., Camargo CA, Anwaruddin S, Baggish AL, Chen AA, Krauser DG, et al. The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. *Am J Cardiol*. 2005;95(8):948-54.
170. Maisel A, Mueller C, Adams K, Jr., Anker SD, Aspromonte N, Cleland JG, et al. State of the art: using natriuretic peptide levels in clinical practice. *Eur J Heart Fail*. 2008;10(9):824-39.

171. Mueller T, Gegenhuber A, Poelz W, Haltmayer M. Diagnostic accuracy of B type natriuretic peptide and amino terminal proBNP in the emergency diagnosis of heart failure. *Heart*. 2005;91(5):606-12.
172. NICE. Chronic heart failure. NICE; 2003.
173. Berger R, Huelsman M, Strecker K, Bojic A, Moser P, Stanek B, et al. B-type natriuretic peptide predicts sudden death in patients with chronic heart failure. *Circulation*. 2002;105(20):2392-7.
174. Gardner RS, Ozalp F, Murday AJ, Robb SD, McDonagh TA. N-terminal pro-brain natriuretic peptide. A new gold standard in predicting mortality in patients with advanced heart failure. *Eur Heart J*. 2003;24(19):1735-43.
175. Koglin J, Pehlivanli S, Schwaiblmair M, Vogeser M, Cremer P, vonScheidt W. Role of brain natriuretic peptide in risk stratification of patients with congestive heart failure. *J Am Coll Cardiol*. 2001;38(7):1934-41.
176. Kubler P, Petruk-Kowalczyk J, Majda J, Reczuch K, Banasiak W, Ponikowski P. Persistent high NTpro-BNP concentration as a negative prognostic factor in patients with decompensated heart failure. *Kardiologia Polska*. 2006;64(3):250-6; discussion 7-8.
177. McDonagh TA, Cunningham AD, Morrison CE, McMurray JJ, Ford I, Morton JJ, et al. Left ventricular dysfunction, natriuretic peptides, and mortality in an urban population. *Heart*. 2001;86(1):21-6.
178. Logeart D, Thabut G, Jourdain P, Chavelas C, Beyne P, Beauvais F, et al. Predischage B-type natriuretic peptide assay for identifying patients at high risk of re-admission after decompensated heart failure. *J Am Coll Cardiol*. 2004;43(4):635-41.
179. Troughton RW, Frampton CM, Yandle TG, Espiner EA, Nicholls MG, Richards AM. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet*. 2000;355(9210):1126-30.

180. Beck-da-Silva L, de Bold A, Fraser M, Williams K, Haddad H. BNP-guided therapy not better than expert's clinical assessment for beta-blocker titration in patients with heart failure. *Congestive heart failure* (Greenwich, Conn). 2005;11(5):248-53; quiz 54-5.
181. Karlström P, Alehagen U, Boman K, Dahlström U, group U-s. Brain natriuretic peptide-guided treatment does not improve morbidity and mortality in extensively treated patients with chronic heart failure: responders to treatment have a significantly better outcome. *Eur J Heart Fail*. 2011;13(10):1096-103.
182. Pfisterer M, Buser P, Rickli H, Gutmann M, Erne P, Rickenbacher P, et al. BNP-guided vs symptom-guided heart failure therapy: the Trial of Intensified vs Standard Medical Therapy in Elderly Patients With Congestive Heart Failure (TIME-CHF) randomized trial. *JAMA*. 2009;301(4):383-92.
183. Lainchbury JG, Troughton RW, Strangman KM, Frampton CM, Pilbrow A, Yandle TG, et al. N-terminal pro-B-type natriuretic peptide-guided treatment for chronic heart failure: results from the BATTLESCARRED (NT-proBNP-Assisted Treatment To Lessen Serial Cardiac Readmissions and Death) trial. *J Am Coll Cardiol*. 2009;55(1):53-60.
184. Persson H, Erntell H, Eriksson B, Johansson G, Swedberg K, Dahlstrom U. Improved pharmacological therapy of chronic heart failure in primary care: a randomized Study of NT-proBNP Guided Management of Heart Failure--SIGNAL-HF (Swedish Intervention study--Guidelines and NT-proBNP AnaLysis in Heart Failure). *Eur J Heart Fail*. 2010;12(12):1300-8.
185. Savarese G, Trimarco B, Dellegrottaglie S, Prastaro M, Gambardella F, Rengo G, et al. Natriuretic peptide-guided therapy in chronic heart failure: a meta-analysis of 2,686 patients in 12 randomized trials. *PloS one*. 2013;8(3):e58287.
186. Porapakham P, Zimmet H, Billah B, Krum H. B-type natriuretic peptide-guided heart failure therapy: A meta-analysis. *Archives of internal medicine*. 2010;170(6):507-14.

187. Felker GM, Hasselblad V, Hernandez AF, O'Connor CM. Biomarker-guided therapy in chronic heart failure: a meta-analysis of randomized controlled trials. *American heart journal*. 2009;158(3):422-30.
188. O'Hanlon R, O'Shea P, Ledwidge M, O'Loughlin C, Lange S, Conlon C, et al. The biologic variability of B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide in stable heart failure patients. *J Card Fail*. 2007;13(1):50-5.
189. Melzi d'Eril G, Tagnochetti T, Nauti A, Klersy C, Papalia A, Vadacca G, et al. Biological variation of N-terminal pro-brain natriuretic peptide in healthy individuals. *Clin Chem*. 2003;49(9):1554-5.
190. Wu AH, Smith A, Wieczorek S, Mather JF, Duncan B, White CM, et al. Biological variation for N-terminal pro- and B-type natriuretic peptides and implications for therapeutic monitoring of patients with congestive heart failure. *Am J Cardiol*. 2003;92(5):628-31.
191. Wu AH. Serial testing of B-type natriuretic peptide and NTpro-BNP for monitoring therapy of heart failure: the role of biologic variation in the interpretation of results. *American heart journal*. 2006;152(5):828-34.
192. Miller WL, Hartman KA, Grill DE, Burnett JC, Jr., Jaffe AS. Only large reductions in concentrations of natriuretic peptides (BNP and NT-proBNP) are associated with improved outcome in ambulatory patients with chronic heart failure. *Clin Chem*. 2009;55(1):78-84.
193. Jougasaki M, Wei CM, Aarhus LL, Heublein DM, Sandberg SM, Burnett JC, Jr. Renal localization and actions of adrenomedullin: a natriuretic peptide. *The American journal of physiology*. 1995;268(4 Pt 2):F657-63.
194. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun*. 1993;192(2):553-60.
195. Jougasaki M, Burnett JC, Jr. Adrenomedullin: potential in physiology and pathophysiology. *Life Sci*. 2000;66(10):855-72.

196. Struck J, Tao C, Morgenthaler NG, Bergmann A. Identification of an Adrenomedullin precursor fragment in plasma of sepsis patients. *Peptides*. 2004;25(8):1369-72.
197. Wilkinson IB, McEniery CM, Bongaerts KH, MacCallum H, Webb DJ, Cockcroft JR. Adrenomedullin (ADM) in the human forearm vascular bed: effect of neutral endopeptidase inhibition and comparison with proadrenomedullin NH2-terminal 20 peptide (PAMP). *British journal of clinical pharmacology*. 2001;52(2):159-64.
198. Nishikimi T, Kitamura K, Saito Y, Shimada K, Ishimitsu T, Takamiya M, et al. Clinical studies on the sites of production and clearance of circulating adrenomedullin in human subjects. *Hypertension*. 1994;24(5):600-4.
199. Sugo S, Minamino N, Kangawa K, Miyamoto K, Kitamura K, Sakata J, et al. Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem Biophys Res Commun*. 1994;201(3):1160-6.
200. Beltowski J, Jamroz A. Adrenomedullin--what do we know 10 years since its discovery? *Polish journal of pharmacology*. 2004;56(1):5-27.
201. Sakai K, Saito K, Ishizuka N. Adrenomedullin synergistically interacts with endogenous vasodilators in rats: a possible role of K(ATP) channels. *European journal of pharmacology*. 1998;359(2-3):151-9.
202. Hayakawa H, Hirata Y, Kakoki M, Suzuki Y, Nishimatsu H, Nagata D, et al. Role of nitric oxide-cGMP pathway in adrenomedullin-induced vasodilation in the rat. *Hypertension*. 1999;33(2):689-93.
203. Wangenstein R, Quesada A, Sainz J, Duarte J, Vargas F, Osuna A. Role of endothelium-derived relaxing factors in adrenomedullin-induced vasodilation in the rat kidney. *European journal of pharmacology*. 2002;444(1-2):97-102.
204. Yang BC, Lipton H, Gumusel B, Hyman A, Mehta JL. Adrenomedullin dilates rat pulmonary artery rings during hypoxia: role of nitric oxide and vasodilator prostaglandins. *Journal of cardiovascular pharmacology*. 1996;28(3):458-62.

205. Shimekake Y, Nagata K, Ohta S, Kambayashi Y, Teraoka H, Kitamura K, et al. Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca²⁺ mobilization, in bovine aortic endothelial cells. *J Biol Chem*. 1995;270(9):4412-7.
206. Nishimatsu H, Suzuki E, Nagata D, Moriyama N, Satonaka H, Walsh K, et al. Adrenomedullin induces endothelium-dependent vasorelaxation via the phosphatidylinositol 3-kinase/Akt-dependent pathway in rat aorta. *Circulation research*. 2001;89(1):63-70.
207. Kohno M, Kano H, Horio T, Yokokawa K, Yasunari K, Takeda T. Inhibition of endothelin production by adrenomedullin in vascular smooth muscle cells. *Hypertension*. 1995;25(6):1185-90.
208. Parkes DG. Cardiovascular actions of adrenomedullin in conscious sheep. *The American journal of physiology*. 1995;268(6 Pt 2):H2574-8.
209. Rademaker MT, Charles CJ, Lewis LK, Yandle TG, Cooper GJ, Coy DH, et al. Beneficial hemodynamic and renal effects of adrenomedullin in an ovine model of heart failure. *Circulation*. 1997;96(6):1983-90.
210. Stangl V, Dschietzig T, Bramlage P, Boye P, Kinkel HT, Staudt A, et al. Adrenomedullin and myocardial contractility in the rat. *European journal of pharmacology*. 2000;408(1):83-9.
211. Szokodi I, Kinnunen P, Tavi P, Weckstrom M, Toth M, Ruskoaho H. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. *Circulation*. 1998;97(11):1062-70.
212. Jougasaki M, Rodeheffer RJ, Redfield MM, Yamamoto K, Wei CM, McKinley LJ, et al. Cardiac secretion of adrenomedullin in human heart failure. *The Journal of clinical investigation*. 1996;97(10):2370-6.
213. Nagaya N, Satoh T, Nishikimi T, Uematsu M, Furuichi S, Sakamaki F, et al. Hemodynamic, renal, and hormonal effects of adrenomedullin infusion in patients with congestive heart failure. *Circulation*. 2000;101(5):498-503.

214. Tsuruda T, Kato J, Kitamura K, Kuwasako K, Imamura T, Koiwaya Y, et al. Adrenomedullin: a possible autocrine or paracrine inhibitor of hypertrophy of cardiomyocytes. *Hypertension*. 1998;31(1 Pt 2):505-10.
215. Lainchbury JG, Troughton RW, Lewis LK, Yandle TG, Richards AM, Nicholls MG. Hemodynamic, hormonal, and renal effects of short-term adrenomedullin infusion in healthy volunteers. *The Journal of clinical endocrinology and metabolism*. 2000;85(3):1016-20.
216. Troughton RW, Frampton CM, Lewis LK, Yandle TG, Richards AM, Nicholls MG. Differing thresholds for modulatory effects of adrenomedullin infusion on haemodynamic and hormone responses to angiotensin II and adrenocorticotrophic hormone in healthy volunteers. *Clinical science (London, England : 1979)*. 2001;101(1):103-9.
217. Nicholls MG. Hemodynamic and hormonal actions of adrenomedullin. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica [et al]*. 2004;37(8):1247-53.
218. Potocki M, Ziller R, Mueller C. Mid-regional pro-adrenomedullin in acute heart failure: a better biomarker or just another biomarker? *Current heart failure reports*. 2012;9(3):244-51.
219. Jougasaki M, Wei CM, McKinley LJ, Burnett JC, Jr. Elevation of circulating and ventricular adrenomedullin in human congestive heart failure. *Circulation*. 1995;92(3):286-9.
220. Nishikimi T, Saito Y, Kitamura K, Ishimitsu T, Eto T, Kangawa K, et al. Increased plasma levels of adrenomedullin in patients with heart failure. *J Am Coll Cardiol*. 1995;26(6):1424-31.
221. Maisel A, Mueller C, Nowak R, Peacock WF, Landsberg JW, Ponikowski P, et al. Mid-region pro-hormone markers for diagnosis and prognosis in acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. *J Am Coll Cardiol*. 2010;55(19):2062-76.
222. Cinar O, Cevik E, Acar A, Kaya C, Ardic S, Comert B, et al. Evaluation of mid-regional pro-atrial natriuretic peptide, procalcitonin, and mid-regional pro-adrenomedullin for the diagnosis and risk stratification of dyspneic ED patients. *The American journal of emergency medicine*. 2012;30(9):1915-20.

223. Maisel A, Mueller C, Nowak RM, Peacock WF, Ponikowski P, Mockel M, et al. Midregion prohormone adrenomedullin and prognosis in patients presenting with acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. *J Am Coll Cardiol*. 2011;58(10):1057-67.
224. von Haehling S, Filippatos GS, Papassotiriou J, Cicoira M, Jankowska EA, Doehner W, et al. Mid-regional pro-adrenomedullin as a novel predictor of mortality in patients with chronic heart failure. *Eur J Heart Fail*. 2010;12(5):484-91.
225. Adlbrecht C, Hulsmann M, Strunk G, Berger R, Mortl D, Struck J, et al. Prognostic value of plasma midregional pro-adrenomedullin and C-terminal-pro-endothelin-1 in chronic heart failure outpatients. *Eur J Heart Fail*. 2009;11(4):361-6.
226. Richards AM, Doughty R, Nicholls MG, MacMahon S, Sharpe N, Murphy J, et al. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: prognostic utility and prediction of benefit from carvedilol in chronic ischemic left ventricular dysfunction. Australia-New Zealand Heart Failure Group. *J Am Coll Cardiol*. 2001;37(7):1781-7.
227. Neuhold S, Huelsmann M, Strunk G, Struck J, Adlbrecht C, Gouya G, et al. Prognostic value of emerging neurohormones in chronic heart failure during optimization of heart failure-specific therapy. *Clin Chem*. 2010;56(1):121-6.
228. Miguel D, Prieto B, Alvarez FV. Biological variation and prognosis usefulness of new biomarkers in liver transplantation. *Clinical chemistry and laboratory medicine : CCLM / FESCC*. 2013;51(6):1241-9.
229. O'Dowd BF, Heiber M, Chan A, Heng HH, Tsui LC, Kennedy JL, et al. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene*. 1993;136(1-2):355-60.
230. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun*. 1998;251(2):471-6.

231. Kleinz MJ, Skepper JN, Davenport AP. Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. *Regul Pept.* 2005;126(3):233-40.
232. Geiger K, Muendlein A, Stark N, Saely CH, Wabitsch M, Fraunberger P, et al. Hypoxia induces apelin expression in human adipocytes. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme.* 2011;43(6):380-5.
233. Melgar-Lesmes P, Pauta M, Reichenbach V, Casals G, Ros J, Bataller R, et al. Hypoxia and proinflammatory factors upregulate apelin receptor expression in human stellate cells and hepatocytes. *Gut.* 2011;60(10):1404-11.
234. Than A, Tee WT, Chen P. Apelin secretion and expression of apelin receptors in 3T3-L1 adipocytes are differentially regulated by angiotensin type 1 and type 2 receptors. *Molecular and cellular endocrinology.* 2012;351(2):296-305.
235. Poirier O, Ciumas M, Eyries M, Montagne K, Nadaud S, Soubrier F. Inhibition of apelin expression by BMP signaling in endothelial cells. *American journal of physiology Cell physiology.* 2012;303(11):C1139-45.
236. Szokodi I, Tavi P, Foldes G, Voutilainen-Myllyla S, Ilves M, Tokola H, et al. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circulation research.* 2002;91(5):434-40.
237. Iwanaga Y, Kihara Y, Takenaka H, Kita T. Down-regulation of cardiac apelin system in hypertrophied and failing hearts: Possible role of angiotensin II-angiotensin type 1 receptor system. *J Mol Cell Cardiol.* 2006;41(5):798-806.
238. Chandrasekaran B, Dar O, McDonagh T. The role of apelin in cardiovascular function and heart failure. *Eur J Heart Fail.* 2008;10(8):725-32.
239. Perjes A, Skoumal R, Tenhunen O, Konyi A, Simon M, Horvath IG, et al. Apelin increases cardiac contractility via protein kinase Cepsilon- and extracellular signal-regulated kinase-dependent mechanisms. *PloS one.* 2014;9(4):e93473.

240. Mahar MA, Rainio A, Ilves M, Lindgren K, Karja-Koskenkari P, Taskinen P, et al. Changes in natriuretic peptides, apelin and adrenomedullin after off-pump and on-pump coronary artery bypass surgery. *J Cardiovasc Surg (Torino)*. 2008;49(6):783-91.
241. Berry MF, Pirolli TJ, Jayasankar V, Burdick J, Morine KJ, Gardner TJ, et al. Apelin has in vivo inotropic effects on normal and failing hearts. *Circulation*. 2004;110(11 Suppl 1):II187-93.
242. Tempel D, de Boer M, van Deel ED, Haasdijk RA, Duncker DJ, Cheng C, et al. Apelin enhances cardiac neovascularization after myocardial infarction by recruiting aplnr+ circulating cells. *Circulation research*. 2012;111(5):585-98.
243. van Kimmenade RR, Januzzi JL, Jr., Ellinor PT, Sharma UC, Bakker JA, Low AF, et al. Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure. *J Am Coll Cardiol*. 2006;48(6):1217-24.
244. Miettinen KH, Magga J, Vuolteenaho O, Vanninen EJ, Punnonen KR, Ylitalo K, et al. Utility of plasma apelin and other indices of cardiac dysfunction in the clinical assessment of patients with dilated cardiomyopathy. *Regul Pept*. 2007;140(3):178-84.
245. Goetze JP, Rehfeld JF, Carlsen J, Videbaek R, Andersen CB, Boesgaard S, et al. Apelin: a new plasma marker of cardiopulmonary disease. *Regul Pept*. 2006;133(1-3):134-8.
246. Ho YL, Lin YH, Lee CM, Hsu RB, Ting HT, Chou NK, et al. Prognostic significance of adipocytokines and extracellular matrix activity in heart failure patients with high B-type natriuretic peptide. *Clin Biochem*. 2009;42(13-14):1407-12.
247. Chen MM, Ashley EA, Deng DX, Tsalenko A, Deng A, Tabibiazar R, et al. Novel role for the potent endogenous inotrope apelin in human cardiac dysfunction. *Circulation*. 2003;108(12):1432-9.
248. Francia P, Salvati A, Balla C, De Paolis P, Pagannone E, Borro M, et al. Cardiac resynchronization therapy increases plasma levels of the endogenous inotrope apelin. *Eur J Heart Fail*. 2007;9(3):306-9.

249. Tominaga S. A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. *FEBS letters*. 1989;258(2):301-4.
250. Trajkovic V, Sweet MJ, Xu D. T1/ST2--an IL-1 receptor-like modulator of immune responses. *Cytokine Growth Factor Rev*. 2004;15(2-3):87-95.
251. Weinberg EO, Shimp M, De Keulenaer GW, MacGillivray C, Tominaga S, Solomon SD, et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation*. 2002;106(23):2961-6.
252. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. 2005;23(5):479-90.
253. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *The Journal of clinical investigation*. 2007;117(6):1538-49.
254. Coglianese EE, Larson MG, Vasan RS, Ho JE, Ghorbani A, McCabe EL, et al. Distribution and clinical correlates of the interleukin receptor family member soluble ST2 in the Framingham Heart Study. *Clin Chem*. 2012;58(12):1673-81.
255. Januzzi JL, Jr., Peacock WF, Maisel AS, Chae CU, Jesse RL, Baggish AL, et al. Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. *J Am Coll Cardiol*. 2007;50(7):607-13.
256. Shimp M, Morrow DA, Weinberg EO, Sabatine MS, Murphy SA, Antman EM, et al. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. *Circulation*. 2004;109(18):2186-90.
257. Weinberg EO, Shimp M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation*. 2003;107(5):721-6.

258. Breidthardt T, Balmelli C, Twerenbold R, Mosimann T, Espinola J, Haaf P, et al. Heart failure therapy-induced early ST2 changes may offer long-term therapy guidance. *J Card Fail.* 2013;19(12):821-8.
259. Boisot S, Beede J, Isakson S, Chiu A, Clopton P, Januzzi J, et al. Serial sampling of ST2 predicts 90-day mortality following destabilized heart failure. *J Card Fail.* 2008;14(9):732-8.
260. Manzano-Fernandez S, Januzzi JL, Pastor-Perez FJ, Bonaque-Gonzalez JC, Boronat-Garcia M, Pascual-Figal DA, et al. Serial monitoring of soluble interleukin family member ST2 in patients with acutely decompensated heart failure. *Cardiology.* 2012;122(3):158-66.
261. Lassus J, Gayat E, Mueller C, Peacock WF, Spinar J, Harjola VP, et al. Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: the Multinational Observational Cohort on Acute Heart Failure (MOCA) study. *International journal of cardiology.* 2013;168(3):2186-94.
262. Ky B, French B, McCloskey K, Rame JE, McIntosh E, Shahi P, et al. High-sensitivity ST2 for prediction of adverse outcomes in chronic heart failure. *Circ Heart Fail.* 2011;4(2):180-7.
263. Gruson D, Lepoutre T, Ahn SA, Rousseau MF. Increased soluble ST2 is a stronger predictor of long-term cardiovascular death than natriuretic peptides in heart failure patients with reduced ejection fraction. *International journal of cardiology.* 2014;172(1):e250-2.
264. Sobczak S, Wojtczak-Soska K, Ciurus T, Sakowicz A, Pietrucha T, Lelonek M. Single ST2 protein measurement predicts adverse outcomes at 1-year follow-up in patients with chronic heart failure. *Polskie Archiwum Medycyny Wewnętrznej.* 2014.
265. Bayes-Genis A, Pascual-Figal D, Januzzi JL, Maisel A, Casas T, Valdes Chavarri M, et al. Soluble ST2 monitoring provides additional risk stratification for outpatients with decompensated heart failure. *Revista espanola de cardiologia.* 2010;63(10):1171-8.
266. Wu AH, Wians F, Jaffe A. Biological variation of galectin-3 and soluble ST2 for chronic heart failure: Implication on interpretation of test results. *American heart journal.* 2013;165(6):995-9.

267. Breidthardt T ST, Drexler B, Noveanu M, Haaf P, Balmelli, Cattelan C IA, Reichlin T, Potocki M, Mueller C. Interleukin family member ST2 rapidly responds to heart failure treatment and its changes predict one-year mortality. *Eur Heart J*. 2011;32(Suppl 1).
268. Sharma UC, Pokharel S, van Brakel TJ, van Berlo JH, Cleutjens JP, Schroen B, et al. Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation*. 2004;110(19):3121-8.
269. Hsu DK, Dowling CA, Jeng KC, Chen JT, Yang RY, Liu FT. Galectin-3 expression is induced in cirrhotic liver and hepatocellular carcinoma. *International journal of cancer Journal international du cancer*. 1999;81(4):519-26.
270. Wang L, Friess H, Zhu Z, Frigeri L, Zimmermann A, Korc M, et al. Galectin-1 and galectin-3 in chronic pancreatitis. *Laboratory investigation; a journal of technical methods and pathology*. 2000;80(8):1233-41.
271. Nishi Y, Sano H, Kawashima T, Okada T, Kuroda T, Kikkawa K, et al. Role of galectin-3 in human pulmonary fibrosis. *Allergology international : official journal of the Japanese Society of Allergology*. 2007;56(1):57-65.
272. Henderson NC, Mackinnon AC, Farnworth SL, Kipari T, Haslett C, Iredale JP, et al. Galectin-3 expression and secretion links macrophages to the promotion of renal fibrosis. *The American journal of pathology*. 2008;172(2):288-98.
273. Hrynchyshyn N, Jourdain P, Desnos M, Diebold B, Funck F. Galectin-3: a new biomarker for the diagnosis, analysis and prognosis of acute and chronic heart failure. *Archives of cardiovascular diseases*. 2013;106(10):541-6.
274. Mueller T, Gegenhuber A, Leitner I, Poelz W, Haltmayer M, Dieplinger B. Diagnostic and prognostic accuracy of galectin-3 and soluble ST2 for acute heart failure. *Clin Chim Acta*. 2016;463:158-64.
275. Lok DJ, Van Der Meer P, de la Porte PW, Lipsic E, Van Wijngaarden J, Hillege HL, et al. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data

from the DEAL-HF study. *Clinical research in cardiology : official journal of the German Cardiac Society*. 2010;99(5):323-8.

276. Lok DJ, Lok SI, Bruggink-Andre de la Porte PW, Badings E, Lipsic E, van Wijngaarden J, et al. Galectin-3 is an independent marker for ventricular remodeling and mortality in patients with chronic heart failure. *Clinical research in cardiology : official journal of the German Cardiac Society*. 2012.

277. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, et al. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *J Am Coll Cardiol*. 2012;60(14):1249-56.

278. de Boer RA, Lok DJ, Jaarsma T, van der Meer P, Voors AA, Hillege HL, et al. Predictive value of plasma galectin-3 levels in heart failure with reduced and preserved ejection fraction. *Annals of medicine*. 2011;43(1):60-8.

279. McCullough PA, Olobatoke A, Vanhecke TE. Galectin-3: a novel blood test for the evaluation and management of patients with heart failure. *Reviews in cardiovascular medicine*. 2011;12(4):200-10.

280. Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR, Januzzi JL. Galectin-3, cardiac structure and function, and long-term mortality in patients with acutely decompensated heart failure. *Eur J Heart Fail*. 2010;12(8):826-32.

281. Milting H, Ellinghaus P, Seewald M, Cakar H, Bohms B, Kassner A, et al. Plasma biomarkers of myocardial fibrosis and remodeling in terminal heart failure patients supported by mechanical circulatory support devices. *The Journal of heart and lung transplantation : the official publication of the International Society for Heart Transplantation*. 2008;27(6):589-96.

282. Anand IS, Rector TS, Kuskowski M, Adourian A, Muntendam P, Cohn JN. Baseline and serial measurements of galectin-3 in patients with heart failure: relationship to prognosis and effect of treatment with valsartan in the Val-HeFT. *Eur J Heart Fail*. 2013;15(5):511-8.

283. Franeckova J, Kubicek Z, Hoskova L, Kotrbata M, Secnik Jr P, Kautzner J, et al. Long-term biological variability of galectin-3 after heart transplantation. *Clinical chemistry and laboratory medicine : CCLM / FESCC*. 2014.
284. Bettencourt P, Ferreira A, Dias P, Pimenta J, Frioies F, Martins L, et al. Predictors of prognosis in patients with stable mild to moderate heart failure. *J Card Fail*. 2000;6(4):306-13.
285. Bouvy ML, Heerdink ER, Leufkens HG, Hoes AW. Predicting mortality in patients with heart failure: a pragmatic approach. *Heart*. 2003;89(6):605-9.
286. Muntwyler J, Abetel G, Gruner C, Follath F. One-year mortality among unselected outpatients with heart failure. *Eur Heart J*. 2002;23(23):1861-6.
287. Scrutinio D, Lagioia R, Ricci A, Clemente M, Boni L, Rizzon P. Prediction of mortality in mild to moderately symptomatic patients with left ventricular dysfunction. The role of the New York Heart Association classification, cardiopulmonary exercise testing, two-dimensional echocardiography and Holter monitoring. *Eur Heart J*. 1994;15(8):1089-95.
288. Klip IT, Voors AA, Anker SD, Hillege HL, Struck J, Squire I, et al. Prognostic value of mid-regional pro-adrenomedullin in patients with heart failure after an acute myocardial infarction. *Heart*. 2011;97(11):892-8.
289. Chandrasekaran B, Kalra PR, Donovan J, Hooper J, Clague JR, McDonagh TA. Myocardial apelin production is reduced in humans with left ventricular systolic dysfunction. *J Card Fail*. 2010;16(7):556-61.
290. Chong KS, Gardner RS, Morton JJ, Ashley EA, McDonagh TA. Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. *Eur J Heart Fail*. 2006;8(4):355-60.
291. Kadoglou NP, Lampropoulos S, Kapelouzou A, Gkontopoulos A, Theofilogiannakos EK, Fotiadis G, et al. Serum levels of apelin and ghrelin in patients with acute coronary syndromes and established coronary artery disease--KOZANI STUDY. *Translational research : the journal of laboratory and clinical medicine*. 2010;155(5):238-46.

292. Weir RA, Chong KS, Dalzell JR, Petrie CJ, Murphy CA, Steedman T, et al. Plasma apelin concentration is depressed following acute myocardial infarction in man. *Eur J Heart Fail.* 2009;11(6):551-8.
293. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Annals of internal medicine.* 2009;150(9):604-12.
294. Program NKDE. Glomerular Filtration Rate (GFR) 2015 [Available from: <http://www.ncbi.nlm.nih.gov/pubmed/>].
295. Bruins S, Fokkema MR, Romer JW, Dejongste MJ, van der Dijs FP, van den Ouweland JM, et al. High intraindividual variation of B-type natriuretic peptide (BNP) and amino-terminal proBNP in patients with stable chronic heart failure. *Clin Chem.* 2004;50(11):2052-8.
296. Khan SQ, O'Brien RJ, Struck J, Quinn P, Morgenthaler N, Squire I, et al. Prognostic value of midregional pro-adrenomedullin in patients with acute myocardial infarction: the LAMP (Leicester Acute Myocardial Infarction Peptide) study. *J Am Coll Cardiol.* 2007;49(14):1525-32.
297. Alehagen U, Dahlstrom U, Rehfeld JF, Goetze JP. Pro-A-type natriuretic peptide, proadrenomedullin, and N-terminal pro-B-type natriuretic peptide used in a multimarker strategy in primary health care in risk assessment of patients with symptoms of heart failure. *J Card Fail.* 2013;19(1):31-9.
298. Gegenhuber A, Struck J, Dieplinger B, Poelz W, Pacher R, Morgenthaler NG, et al. Comparative evaluation of B-type natriuretic peptide, mid-regional pro-A-type natriuretic peptide, mid-regional pro-adrenomedullin, and Copeptin to predict 1-year mortality in patients with acute destabilized heart failure. *J Card Fail.* 2007;13(1):42-9.
299. Masson S, Latini R, Carbonieri E, Moretti L, Rossi MG, Ciricugno S, et al. The predictive value of stable precursor fragments of vasoactive peptides in patients with chronic heart failure: data from the GISSI-heart failure (GISSI-HF) trial. *Eur J Heart Fail.* 2010;12(4):338-47.
300. Funke-Kaiser A, Mann K, Colquhoun D, Zeller T, Hunt D, Simes J, et al. Midregional proadrenomedullin and its change predicts recurrent major coronary events and heart failure in

stable coronary heart disease patients: the LIPID study. *International journal of cardiology*. 2014;172(2):411-8.

301. Travaglini F, Russo V, De Berardinis B, Numeroso F, Catania P, Cervellin G, et al. Thirty and ninety days mortality predictive value of admission and in-hospital procalcitonin and mid-regional pro-adrenomedullin testing in patients with dyspnea. Results from the VErifying DYspnea trial. *The American journal of emergency medicine*. 2014;32(4):334-41.

302. Breidthardt T, Mebazaa A, Mueller CE. Predicting progression in nondiabetic kidney disease: the importance of cardiorenal interactions. *Kidney Int*. 2009;75(3):253-5.

303. Fallah-Rad N, Walker JR, Wassef A, Lytwyn M, Bohonis S, Fang T, et al. The utility of cardiac biomarkers, tissue velocity and strain imaging, and cardiac magnetic resonance imaging in predicting early left ventricular dysfunction in patients with human epidermal growth factor receptor II-positive breast cancer treated with adjuvant trastuzumab therapy. *J Am Coll Cardiol*. 2011;57(22):2263-70.

304. Lawley C, Wainwright C, Segelov E, Lynch J, Beith J, McCrohon J. Pilot study evaluating the role of cardiac magnetic resonance imaging in monitoring adjuvant trastuzumab therapy for breast cancer. *Asia-Pacific journal of clinical oncology*. 2012;8(1):95-100.

305. Suzuki Y, Itoh H, Katagiri F, Sato F, Kawasaki K, Sato Y, et al. Significant decrease in plasma midregional proadrenomedullin level in patients with end-stage renal disease after living kidney transplantation. *Peptides*. 2013;43:102-4.

306. Artunc F, Nowak A, Mueller C, Breidthardt T, Twerenbold R, Wagner R, et al. Plasma concentrations of the vasoactive peptide fragments mid-regional pro-adrenomedullin, C-terminal pro-endothelin 1 and copeptin in hemodialysis patients: associated factors and prediction of mortality. *PloS one*. 2014;9(1):e86148.

307. Peckova M, Charvat J, Schuck O, Zamrazil V, Bilek R, Hill M, et al. Plasma adrenomedullin and subclinical cardiorenal syndrome in patients with type 2 diabetes mellitus. *J Int Med Res*. 2012;40(4):1552-9.

308. Rademaker MT, Charles CJ, Nicholls MG, Richards AM. Haemodynamic, endocrine and renal actions of adrenomedullin 5 in an ovine model of heart failure. *Clinical science (London, England : 1979)*. 2012;122(9):429-37.
309. Damman K, Navis G, Voors AA, Asselbergs FW, Smilde TD, Cleland JG, et al. Worsening renal function and prognosis in heart failure: systematic review and meta-analysis. *J Card Fail*. 2007;13(8):599-608.
310. Damman K, Testani JM. The kidney in heart failure: an update. *Eur Heart J*. 2015.
311. de Silva R, Nikitin NP, Witte KK, Rigby AS, Goode K, Bhandari S, et al. Incidence of renal dysfunction over 6 months in patients with chronic heart failure due to left ventricular systolic dysfunction: contributing factors and relationship to prognosis. *Eur Heart J*. 2006;27(5):569-81.
312. Khan NA, Ma I, Thompson CR, Humphries K, Salem DN, Sarnak MJ, et al. Kidney function and mortality among patients with left ventricular systolic dysfunction. *Journal of the American Society of Nephrology : JASN*. 2006;17(1):244-53.
313. Loffler AI, Cappola TP, Fang J, Hetzel SJ, Kadlec A, Astor B, et al. Effect of renal function on prognosis in chronic heart failure. *Am J Cardiol*. 2015;115(1):62-8.
314. Maeder MT, Rickli H, Pfisterer ME, Muzzarelli S, Ammann P, Fehr T, et al. Incidence, clinical predictors, and prognostic impact of worsening renal function in elderly patients with chronic heart failure on intensive medical therapy. *American heart journal*. 2012;163(3):407-14, 14 e1.
315. Zhang W, Wen D, Zou YF, Shen PY, Xu YW, Shi H, et al. One-year survival and renal function recovery of acute kidney injury patients with chronic heart failure. *Cardiorenal Med*. 2015;5(1):40-7.
316. Foldes G, Horkay F, Szokodi I, Vuolteenaho O, Ilves M, Lindstedt KA, et al. Circulating and cardiac levels of apelin, the novel ligand of the orphan receptor APJ, in patients with heart failure. *Biochem Biophys Res Commun*. 2003;308(3):480-5.
317. Mullens W, Bartunek J, Wilson Tang WH, Delrue L, Herbots L, Willems R, et al. Early and late effects of cardiac resynchronization therapy on force-frequency relation and contractility

regulating gene expression in heart failure patients. *Heart rhythm : the official journal of the Heart Rhythm Society*. 2008;5(1):52-9.

318. Wang W, McKinnie SM, Patel VB, Haddad G, Wang Z, Zhabyeyev P, et al. Loss of Apelin exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: therapeutic potential of synthetic Apelin analogues. *J Am Heart Assoc*. 2013;2(4):e000249.

319. Dalzell JR, Jackson CE, Chong KS, McDonagh TA, Gardner RS. Do plasma concentrations of apelin predict prognosis in patients with advanced heart failure? *Biomarkers in medicine*. 2014;8(6):807-13.

320. Sato T, Suzuki T, Watanabe H, Kadowaki A, Fukamizu A, Liu PP, et al. Apelin is a positive regulator of ACE2 in failing hearts. *The Journal of clinical investigation*. 2013;123(12):5203-11.

321. Pascual-Figal DA, Ordonez-Llanos J, Tornel PL, Vazquez R, Puig T, Valdes M, et al. Soluble ST2 for predicting sudden cardiac death in patients with chronic heart failure and left ventricular systolic dysfunction. *J Am Coll Cardiol*. 2009;54(23):2174-9.

322. Shamim W, Yousufuddin M, Cicoria M, Gibson DG, Coats AJS, Henein MY. Incremental changes in QRS duration in serial ECGs over time identify high risk elderly patients with heart failure. *Heart*. 2002;88(1):47-51.

323. Ciccone MM, Cortese F, Gesualdo M, Riccardi R, Di Nunzio D, Moncelli M, et al. A novel cardiac bio-marker: ST2: a review. *Molecules (Basel, Switzerland)*. 2013;18(12):15314-28.

324. Bao YS, Na SP, Zhang P, Jia XB, Liu RC, Yu CY, et al. Characterization of interleukin-33 and soluble ST2 in serum and their association with disease severity in patients with chronic kidney disease. *Journal of clinical immunology*. 2012;32(3):587-94.

325. Bayes-Genis A, Zamora E, de Antonio M, Galan A, Vila J, Urrutia A, et al. Soluble ST2 serum concentration and renal function in heart failure. *J Card Fail*. 2013;19(11):768-75.

326. Newby LK, Storrow AB, Gibler WB, Garvey JL, Tucker JF, Kaplan AL, et al. Bedside multimarker testing for risk stratification in chest pain units: The chest pain evaluation by creatine kinase-MB, myoglobin, and troponin I (CHECKMATE) study. *Circulation*. 2001;103(14):1832-7.

327. Sabatine MS, Morrow DA, de Lemos JA, Gibson CM, Murphy SA, Rifai N, et al. Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. *Circulation*. 2002;105(15):1760-3.
328. Westerhout CM, Fu Y, Lauer MS, James S, Armstrong PW, Al-Hattab E, et al. Short- and long-term risk stratification in acute coronary syndromes: the added value of quantitative ST-segment depression and multiple biomarkers. *J Am Coll Cardiol*. 2006;48(5):939-47.
329. Eapen DJ, Manocha P, Patel RS, Hammadah M, Veledar E, Wassel C, et al. Aggregate risk score based on markers of inflammation, cell stress, and coagulation is an independent predictor of adverse cardiovascular outcomes. *J Am Coll Cardiol*. 2013;62(4):329-37.
330. Wilson PW. Prediction of cardiovascular disease events. *Cardiology clinics*. 2011;29(1):1-13.
331. Potocki M, Breidthardt T, Reichlin T, Morgenthaler NG, Bergmann A, Noveanu M, et al. Midregional pro-adrenomedullin in addition to b-type natriuretic peptides in the risk stratification of patients with acute dyspnea: an observational study. *Critical care (London, England)*. 2009;13(4):R122.
332. Ralli S, Horwich TB, Fonarow GC. Relationship between anemia, cardiac troponin I, and B-type natriuretic peptide levels and mortality in patients with advanced heart failure. *American heart journal*. 2005;150(6):1220-7.
333. van Kimmenade RR, Januzzi JL, Jr., Baggish AL, Lainchbury JG, Bayes-Genis A, Richards AM, et al. Amino-terminal pro-brain natriuretic Peptide, renal function, and outcomes in acute heart failure: redefining the cardiorenal interaction? *J Am Coll Cardiol*. 2006;48(8):1621-7.
334. Allen LA, Felker GM. Multi-marker strategies in heart failure: clinical and statistical approaches. *Heart failure reviews*. 2010;15(4):343-9.
335. Rehman SU, Martinez-Rumayor A, Mueller T, Januzzi JL, Jr. Independent and incremental prognostic value of multimarker testing in acute dyspnea: results from the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) study. *Clin Chim Acta*. 2008;392(1-2):41-5.

336. Ingle L, Cleland JG, Clark AL. The relation between repeated 6-minute walk test performance and outcome in patients with chronic heart failure. *Annals of physical and rehabilitation medicine*. 2014;57(4):244-53.
337. Tabata M, Shimizu R, Kamekawa D, Kato M, Kamiya K, Akiyama A, et al. Six-minute walk distance is an independent predictor of hospital readmission in patients with chronic heart failure. *International heart journal*. 2014;55(4):331-6.
338. Wegrzynowska-Teodorczyk K, Rudzinska E, Lazarczyk M, Nowakowska K, Banasiak W, Ponikowski P, et al. Distance covered during a six-minute walk test predicts long-term cardiovascular mortality and hospitalisation rates in men with systolic heart failure: an observational study. *Journal of physiotherapy*. 2013;59(3):177-87.
339. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. *Clin Chem*. 2005;51(10):1823-9.
340. Caruhel P, Mazier C, Kunde J, Morgenthaler NG, Darbouret B. Homogeneous time-resolved fluoroimmunoassay for the measurement of midregional proadrenomedullin in plasma on the fully automated system B.R.A.H.M.S KRYPTOR. *Clin Biochem*. 2009;42(7-8):725-8.
341. Azizi M, Iturrioz X, Blanchard A, Peyrard S, De Mota N, Chartrel N, et al. Reciprocal regulation of plasma apelin and vasopressin by osmotic stimuli. *Journal of the American Society of Nephrology : JASN*. 2008;19(5):1015-24.
342. De Mota N, Reaux-Le Goazigo A, El Messari S, Chartrel N, Roesch D, Dujardin C, et al. Apelin, a potent diuretic neuropeptide counteracting vasopressin actions through inhibition of vasopressin neuron activity and vasopressin release. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101(28):10464-9.
343. Maguire JJ, Kleinz MJ, Pitkin SL, Davenport AP. [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. *Hypertension*. 2009;54(3):598-604.

344. Mesmin C, Dubois M, Becher F, Fenaille F, Ezan E. Liquid chromatography/tandem mass spectrometry assay for the absolute quantification of the expected circulating apelin peptides in human plasma. *Rapid communications in mass spectrometry : RCM*. 2010;24(19):2875-84.
345. Mesmin C, Fenaille F, Becher F, Tabet JC, Ezan E. Identification and characterization of apelin peptides in bovine colostrum and milk by liquid chromatography-mass spectrometry. *Journal of proteome research*. 2011;10(11):5222-31.
346. Zhen EY, Higgs RE, Gutierrez JA. Pyroglutamyl apelin-13 identified as the major apelin isoform in human plasma. *Analytical biochemistry*. 2013;442(1):1-9.
347. Mueller T, Zimmermann M, Dieplinger B, Ankersmit HJ, Haltmayer M. Comparison of plasma concentrations of soluble ST2 measured by three different commercially available assays: the MBL ST2 assay, the Presage ST2 assay, and the R&D ST2 assay. *Clin Chim Acta*. 2012;413(19-20):1493-4.
348. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, et al. Executive summary: heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation*. 2010;121(7):948-54.
349. Neumann T, Biermann J, Erbel R, Neumann A, Wasem J, Ertl G, et al. Heart failure: the commonest reason for hospital admission in Germany: medical and economic perspectives. *Deutsches Arzteblatt international*. 2009;106(16):269-75.
350. Bocchi EA, Arias A, Verdejo H, Diez M, Gomez E, Castro P. The reality of heart failure in Latin America. *J Am Coll Cardiol*. 2013;62(11):949-58.
351. Heidenreich PA, Albert NM, Allen LA, Bluemke DA, Butler J, Fonarow GC, et al. Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association. *Circ Heart Fail*. 2013;6(3):606-19.
352. Blair JE, Huffman M, Shah SJ. Heart failure in North America. *Current cardiology reviews*. 2013;9(2):128-46.

353. Schou M, Gustafsson F, Videbaek L, Tuxen C, Keller N, Handberg J, et al. Extended heart failure clinic follow-up in low-risk patients: a randomized clinical trial (NorthStar). *Eur Heart J*. 2013;34(6):432-42.
354. Luttik ML, Brons M, Jaarsma T, Hillege HL, Hoes A, de Jong R, et al. Design and methodology of the COACH-2 (Comparative study on guideline adherence and patient compliance in heart failure patients) study: HF clinics versus primary care in stable patients on optimal therapy. *Netherlands heart journal : monthly journal of the Netherlands Society of Cardiology and the Netherlands Heart Foundation*. 2012;20(7-8):307-12.
355. Anguita M, Esteban F, Castillo JC, Mazuelos F, Lopez-Granados A, Arizon JM, et al. [Usefulness of brain natriuretic peptide levels, as compared with usual clinical control, for the treatment monitoring of patients with heart failure]. *Medicina clinica*. 2010;135(10):435-40.
356. Wu AH, Smith A. Biological variation of the natriuretic peptides and their role in monitoring patients with heart failure. *Eur J Heart Fail*. 2004;6(3):355-8.
357. Zhang R, Zhang Y, An T, Guo X, Yin S, Wang Y, et al. Prognostic value of sST2 and galectin-3 for death relative to renal function in patients hospitalized for heart failure. *Biomarkers in medicine*. 2015;9(5):433-41.
358. Peacock WF, Nowak R, Christenson R, DiSomma S, Neath SX, Hartmann O, et al. Short-term mortality risk in emergency department acute heart failure. *Acad Emerg Med*. 2011;18(9):947-58.
359. O'Seaghdha CM, Hwang SJ, Ho JE, Vasan RS, Levy D, Fox CS. Elevated galectin-3 precedes the development of CKD. *Journal of the American Society of Nephrology : JASN*. 2013;24(9):1470-7.
360. Tang WH, Shrestha K, Shao Z, Borowski AG, Troughton RW, Thomas JD, et al. Usefulness of plasma galectin-3 levels in systolic heart failure to predict renal insufficiency and survival. *Am J Cardiol*. 2011;108(3):385-90.
361. Zamora E, Lupon J, de Antonio M, Galan A, Domingo M, Urrutia A, et al. Renal function largely influences Galectin-3 prognostic value in heart failure. *International journal of cardiology*. 2014;177(1):171-7.

362. Mueller T, Leitner I, Egger M, Haltmayer M, Dieplinger B. Association of the biomarkers soluble ST2, galectin-3 and growth-differentiation factor-15 with heart failure and other non-cardiac diseases. *Clin Chim Acta*. 2015;445:155-60.
363. Kronenberg F. Emerging risk factors and markers of chronic kidney disease progression. *Nat Rev Nephrol*. 2009;5(12):677-89.